Pharmacologically active compounds in the environment and their chirality

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Abstract

Pharmacologically active compounds including both legally used pharmaceuticals and illicit drugs are potent environmental contaminants. Extensive research has been undertaken over the recent years to understand their environmental fate and toxicity. The one very important phenomenon that has been overlooked by environmental researchers studying the fate of pharmacologically active compounds in the environment is their chirality. Chiral drugs can exist in the form of enantiomers, which have similar physicochemical properties but differ in their biological properties such as distribution, metabolism and excretion, as these processes (due to stereospecific interactions of enantiomers with biological systems) usually favour one enantiomer over the other. Additionally, due to different pharmacological activity, enantiomers of chiral drugs can differ in toxicity. Furthermore, degradation of chiral drugs during wastewater treatment and in the environment can be stereoselective and can lead to chiral products of varied toxicity. The distribution of different enantiomers of the same chiral drug in the aquatic environment and biota can also be stereoselective. Biological processes can lead to stereoselective enrichment or depletion of the enantiomeric composition of chiral drugs. As a result the very same drug might reveal different activity and toxicity and this will depend on its origin and exposure to several factors governing its fate in the environment.

In this review a discussion of the importance of chirality of pharmacologically active compounds in the environmental context is undertaken and suggestions for directions in further research are made. Several groups of chiral drugs of major environmental relevance are discussed and their pharmacological action and disposition in the body is also outlined as it is a key factor in developing a full understanding of their environmental occurrence, fate and toxicity.

This review will be of interest to environmental scientists, especially those interested in issues associated with environmental contamination with pharmacologically active compounds and chiral pollutants. As the review will outline current state of knowledge on chiral drugs, it will be of value to anyone interested in the phenomenon of chirality, chiral drugs, their stereoselective disposition in the body and environmental fate.

Keywords: chirality, chiral drugs, pharmaceuticals, illicit drugs, environment

1. Introduction

Pharmacologically active compounds that include both legally used pharmaceuticals and illicit drugs are a group of emerging environmental contaminants, potentially hazardous compounds that have been receiving steadily growing attention over the last decade. Surprisingly, there are limited data and minimal understanding of the environmental occurrence, transport, fate and exposure for many pharmaceuticals and their metabolites, despite their frequently high annual usage[1-4]. Some of the most commonly used pharmaceuticals are sold in the UK in hundreds of tonnes per year. Usage of drugs is going to increase in the future due to the ageing population in western countries and an increase in consumption levels in the developing world. Illicit drugs, belonging to the same group of biologically active compounds, have however hardly been studied in the environment[5-9]. One of the reasons for a lack of data was, until recently, a lack of suitable analytical methods capable of detecting polar compounds at very low concentrations in a complex environmental matrix. However, due to increasing concern regarding the possible effect of pharmaceuticals on humans and wildlife, an increase in interest in the environmental occurrence of these compounds is to be expected.

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There is also a general lack of information concerning eco-toxicological data on pharmaceuticals and their metabolites. Although the preliminary aquatic risk assessment of pharmaceuticals indicate that exposure concentrations are one to two orders of magnitude lower than LOEC (lowest observed effect concentration) and NOEC (no observed effect concentration) values suggesting low risk, long-term environmental risks associated with the presence of pharmaceuticals are hardly known. For example a decline of vulture population in Pakistan due to exposure to low levels of diclofenac proves that the presence of pharmaceuticals in the environment cannot be underestimated[10]. Therefore, when discussing toxicity results obtained with traditional toxicity testing procedures such as lethality, growth and reproduction, it is important to consider that these results do not represent the true potential hazard of pharmacologically active compounds in the environment, due to the duration of these procedures versus actual environmental exposure

The aim of this review is to outline the state of knowledge and future research directions concerning environmentally relevant pharmacologically active compounds which reveal chiral nature. Several themes are discussed:

- Sources, distribution and occurrence of pharmacologically active compounds in the environment.
- Principles of chirality and its importance in the disposition of chiral drugs in humans.
- Current state of knowledge concerning occurrence, fate and toxicity of chiral drugs in the environment.
- Review of major groups of chiral drugs of environmental concern including their pharmacokinetics and environmental fate and toxicity.

This review is written by an environmental chemist and directed mainly at environmental scientists and therefore only certain aspects of pharmacological action and disposition of chiral drugs in the body relevant to the environmental field are outlined as they are key factors in developing a full understanding of environmental occurrence, fate and toxicity of chiral drugs.

2. Pharmacologically active compounds in the environment

2.1. Sources and distribution of pharmaceuticals and illicit drugs in the environment

Pharmaceuticals and illicit drugs enter the aquatic environment mainly through treated (or raw) sewage from domestic households and hospitals, waste effluents from manufacturing processes and runoff. Domestic animals are the main direct source of the environmental disposal of many veterinary pharmaceuticals (antibiotics, anaesthetics, etc), as manure is very often applied to agricultural fields as a fertiliser. Sludge from wastewater plants containing human pharmaceuticals (especially those of more hydrophobic nature) is also used as fertiliser in agricultural fields or transported to landfill. Pharmaceuticals might enter the aqueous environment as parent unaltered compounds, metabolites, conjugates, or might undergo transformation during wastewater treatment to produce compounds of significant concern to humans and wildlife. Many of these compounds are ubiquitous and persistent in the environment. Additionally, they are continuously introduced into the environment; therefore even compounds of a low persistence might cause adverse effects. The other issue is the synergistic effect of different pharmaceuticals on organisms, through their combined parallel action. Due to their very often polar and non-volatile nature, many pharmaceuticals will not undergo volatilisation from the aqueous environment, which extends the exposure of aquatic organisms to these compounds. Aquatic organisms are an obvious primary target. However, the terrestrial environment is also at risk[1, 2, 11, 12]. Pharmaceuticals have been also detected in drinking water, which poses a direct risk to humans[2] and raises the issue of contaminated water sources and especially water reuse.

2.2. Occurrence of pharmaceuticals and illicit drugs in the environment

Pharmaceuticals represent a versatile group of compounds, which are found in surface waters at the levels of up to a few $\mu g\ L^{-1}$ [1, 2, 8, 9, 13-15]. Thousands of pharmaceuticals are approved for human or

veterinary usage, although only a very small percentage of these compounds have been studied for presence in the environment (about 80-150 pharmaceuticals)[2, 16], not to mention their active metabolites and degradation products. Antibiotics, steroid compounds and analgesics/anti-inflammatory drugs are the most widely studied pharmaceuticals. These compounds are widely used not only in human therapy but also in animal treatment. A huge percentage of antibiotics such as doxycycline, oxytetracycline and levofloxacin is excreted by the human body unchanged. Moreover, due to their direct influence on the natural microbiota and the formation of resistant strains, the risk concerning their usage is significant[1, 4, 13, 16]. Anti-inflammatories (diclofenac, ibuprofen, naproxen, ketoprofen), blood lipid regulators and their metabolites (gemfibrozil and clofibric acid) were recently found to be toxic in respect of certain bacteria and algae[13]. Additionally, some of them, such as diclofenac, are poorly removed by WWTP (wastewater treatment plant), ubiquitous and persistent in the environment[1, 9]. Antiepileptic drugs are also ubiquitous, poorly removed in WWTP and toxic to bacteria and algae[1,18]. Carbamazepine has been widely detected in the environment, even if excreted at a low percentage as an unchanged drug (3%)[1, 2, 8, 9].

For several groups of pharmaceuticals of a very high usage, there is little or no data on their presence and fate in the environment and effects on non-targeted organisms. These are for example central nervous system drugs such as: antipsychotic drugs, antidepressants or sedative drugs which are distributed in huge quantities across the world. For example popular antidepressants such as venlafaxine, fluoxetine or citalopram are prescribed in England in tens of tonnes annually[19]. Surprisingly, despite their possible physiologic effect on non-targeted aqueous organisms, their presence has not been widely analysed in the environment. Antineoplastics used in hospitals as chemotherapy agents are suspected of potential mutagenic, teratogenic or carcinogenic effects on non-targeted aqueous organisms. Some, such as phosphamide, are poorly removed from WWTP, although there is minimal knowledge about their overall stability during wastewater treatment and their fate in the environment[1].

Illicit drugs have also hardly been studied in the environment and only a few reports have been published on the occurrence of these compounds in surface water and/or wastewater. Investigations have taken place in the following countries: Italy[5, 20], Spain[21-24], Ireland[25], UK[5, 8, 9], Belgium[7], Switzerland[26] and the USA[27-29]. Due to the limited extent of research undertaken in this field, there is minimal understanding of the environmental occurrence, transport, fate and exposure for these compounds and their very often active metabolites. There is also no information available on the ecotoxicity of illicit drugs and their metabolites. Although illicit drugs are present in the aquatic environment at low ppt levels, their possible effect on living organisms cannot be overestimated. This is because illicit drugs reveal very high pharmacological potency in humans at very low levels. For example, LSD is among the most potent drugs known, being active in humans at doses from about 20 μg[30].

Although several projects concerning the presence and fate of pharmaceuticals have been carried out across the world in recent years they have usually concentrated on a limited number of pharmaceuticals. Additionally, only a very limited, if any, investigation into the presence of their metabolites has been undertaken despite the fact that analysis of pharmaceuticals' transformation products is a crucial factor in understanding their fate and effects in the environment, especially because many metabolites of pharmaceuticals are biologically active. The verification of environmental levels of pharmacologically active compounds and their removal very often does not take into consideration conjugated forms of studied drugs, which might result in an underestimation of environmental exposure. The one very important phenomenon that was hugely overlooked by environmental researchers studying the fate of pharmaceuticals and illicit drugs in the environment is their chirality.

Lack of interest of environmental researchers in the chirality of drugs is surprising as chirality of several other environmental pollutants was widely studied[31-34]. Among them are: phenoxyalkanoic acid herbicides, acetamide pesticides, organophosphorous compounds, pyrethroids, polychlorinated

biphenyls (PCBs), polychlorinated dibenzo-p-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs). Biotransformation in animals, degradation, bioaccumulation, and toxicity of these compounds are often stereospecific[35]. For example enantioselectivity in microbial mediated biotransformation phenoxyalkanoic herbicides such as: mecoprop and dichlorprop (of which only *R*-enantiomer has herbicidal activity) is widely reported. Stereospecific biodegradation of acetamide pesticides such as metolachlor and metalaxyl is also understood. Stereospecific toxicokinetics was observed in the case of pyrethroids in mammals. Stereoselectivity of chiral metabolites of PCBs, PCB-methyl sulphones ($MeSO_2$-PCBs) in mammalian species was also reported. It was observed that (+)-fenamiphos (organophosphorous pesticide) was more toxic to daphnids and also dissipated from soils faster than its antipode. In the case of racemic ruelene, change in enantioselectivity was observed as a result of temperature changes and deforestation, which might have huge implications in a changing climate[31, 32].

3. Chiral drugs

3.1. Principles of chirality

Chirality plays an important role in the life of plants and animals but it is also vital in the agricultural, pharmaceutical and chemical industries. All proteins, enzymes and carbohydrates are chiral. More than half of the drugs currently in use are chiral compounds and many of these are marketed as racemates consisting of an equimolar mixture of two enantiomers. Chiral natural compounds, as opposed to many chiral man-made chemicals, exist in one enantiomeric form (e.g. amino-acids are *l*-isomers and natural sugars are *d*-isomers). Enantiomers of the same drug have similar physicochemical properties but differ in their biological properties[36]. Distribution, metabolism and excretion in the body usually favour one enantiomer over the other. This results from the fact that enantiomers stereoselectively react in biological systems for example with enzymes. Plasma protein binding is also stereoselective. Furthermore, biological transformation of drugs can be steroselective, so the enantiomeric composition of chiral compounds may be changed. Metabolites of achiral compounds can also be chiral (e.g. achiral albendazole or risperidone are transformed into chiral metabolites). Additionally, due to different pharmacological activity, chiral drugs can differ in toxicity. Thalidomide is an excellent example. As a result of the administration of the racemic form of this sedative drug to pregnant woman, thousands of babies were born with deformities in 1960s. A therapeutic (+)-enantiomer of thalidomide is harmless (has tranquilising properties) but its (-)-enantiomer is teratogenic and leads to malformations of embryos if administered to pregnant woman. Furthermore, (+)-enantiomer in the human body undergoes *in vivo* inter-conversion leading to toxic (-)-enantiomer. Therefore even administration of harmless (+)-enantiomer might lead to serious consequences.

Unfortunately, many chiral drugs are still produced as racemate because either their chiral separation is difficult, or the cost of their stereoselective synthesis is too high, or simply at the time of the discovery of the drug, only racemic mixture was considered in the animal and the clinical pharmacology, toxicology and teratology studies and knowledge of pharmacodynamic, pharmacokinetic or toxicological properties of individual enantiomers is still limited[37, 38].

3.2. Global market

The phenomenon of chirality is currently a major driving force in drug discovery and development. In the past decades chiral drugs were produced mainly as racemates but with the introduction of new technologies allowing for the separation of optical isomers an interest in the design and distribution of only one active enantiomer significantly increased. The rationale behind administration of one active enantiomer only is obvious as it leads to: simplification of the interpretation of the basic pharmacology, therapeutic and toxic effects, pharmacokinetic properties and the relationship of plasma concentrations to effects. Other advantages include: possibility of administration of lower dosages of drugs, reduced drug interactions and toxicity[38-41].

Worldwide sales of chiral drugs in single-enantiomer form continuously increase. However, a substantial quantity of chiral drugs is still sold in the racemic form. The worldwide market share of single enantiomer form increased from 27% in 1996 to 39% in 2002[39]. Shimazawa et al.[42] observed a similar trend in Japan. Murakami[43] published the most recent report on chiral synthetic drugs launched over the last 20 years. Tab. 1 represents new synthetic drugs introduced worldwide between the years 1985 and 2004[43].

There is at present a tendency in the pharmaceutical industry to switch from racemates to single enantiomers. This is because in 1992 FDA[44] and in 1994 EU[45] issued guidelines concerning the development of new chiral drugs, which favour the development of single-enantiomer rather than racemic form[40, 46, 47]. Among drugs for which a racemic switch was undertaken are: *R*-verapamil, *S*-fluoxetine, *S*-ketoprofen, *R*-albuterol, levofloxacin, esoprazole, etc (Tab. 2). It has to be however remembered that sometimes racemic switch does not lead to the expected increase of drug potency and/or in some cases the single-enantiomer form might be less safe than racemic form of the same drug (e.g. fluoxetine, labetalol, propanolol and sotalol). This phenomenon can be explained by direct pharmacodynamic or pharmacokinetic competition/interaction between two enantiomers which results in the prevention of one enantiomer toxicity by another (e.g. labetalol) or a specific protective effect provided by one of the enantiomers in the racemic mixture[40, 46]. Propranolol for example was found to show lower beta-blocking activity if introduced as *S*(-)-enantiomer. This suggests that the presence of *R*(+)-propranolol has the beneficial effect of *S*(-)-propranolol availability[37, 46]. Dilevalol, on the other hand, the beta-blocking stereoisomer of labetalol was withdrawn from the market due to its increased hepatoxicity when compared to the racemic mixture[38]. Sotalol is another chiral drug that is used as a racemate despite initial trials evaluating the usage of only (+)-enantiomer as an anti-arrhythmic agent[38]. Therefore despite considerable interest in the usage of only one active enantiomer, many chiral drugs are still used in racemic form. This is of great importance and can have huge implications when the environmental fate and ecotoxicity of chiral drugs are considered.

3.3. Chiral drugs in humans

The phenomenon of chirality exists in all biological systems. Biomacromolecules (e.g. enzymes) composed from chiral subunits (e.g. amino acids) are capable of selective recognition and transformation of individual stereoisomers of other chiral molecules. Stereoselective interactions of biomolecules with chiral molecules are an essential part of all vital biological processes including disposition of chiral drugs and their pharmacological activity. Therefore enantiomers of chiral drugs have to be treated as independent entities rather than just different forms of the same drug as one enantiomer may produce the desired therapeutic activity, while the other might be inactive or toxic[36, 38, 40, 41, 48].

Chiral drugs produced in their racemic form can be divided into three groups[36, 41, 49]: drugs with only one major bioactive enantiomer, drugs with two bioactive enantiomers and drugs with only one major bioactive enantiomer but with the potential for the second non-active enantiomer to be transformed in the body to its bioactive form through chiral inversion.

3.3.1. Drugs with only one major bioactive enantiomer

The majority of chiral drugs belong to this group. These drugs are usually distributed as racemates. Among beta-blockers and calcium channel blockers, levorotary isomer is more active than dextrorotary isomer (e.g. *S*(-)-propranolol is 100 times more active than *R*(+)-propranolol). Sympathomimetic drug-selective beta-aderenoceptor antagonists such as sabutamol have *l*-enantiomer (*R*(-)-enantiomer) which is pharmacologically active but *d*-enantiomer can be responsible for some side-effects. In the case of hypnotics such as barbiturates, only their *R*(-)-isomer is hypnotic/sedative and *S*(+)-isomer will be either inactive or excitative (convulsant). *R*(-)-methadone is about 25-50 times more potent than *S*(+)-methadone. On the other hand antidepressant *S*(+)-citalopram is 100 times more potent than its *R*(-)-enantiomer. Other chiral drugs belonging to this group include: antibiotics (e.g. ofloxacin), anti-inflammatory/analgesics

(e.g. ketoprofen) and psycho-stimulants (e.g. amphetamines)[36]. The phenomenon of amphetamine's chirality is crucial in forensic analysis. *S*(+)-enantiomer of amphetamine is known to be much more potent than *R*(-)-enantiomer and is present in illicitly used amphetamine. On the other hand *R*(-)-enantiomer is often present in pharmaceutical preparations and/or excreted as a metabolite of certain drugs (e.g. selegiline).

Drugs with only one major bioactive enantiomer can be divided into two groups:

- Stereoisomers that have the same quality of action but differ in potency

Calcium channel blockers distributed in the racemic form belong to this group (with the exception of achiral nifedipine, and diltiazem, which is sold in the form of one active *cis* (+)-stereoisomer). These drugs are characterised by quantitative differences in potency, rather then in pharmacological effects elicited (e.g. warfarin and verapamil)[49, 50, 53]. For example *S*-verapamil is known to be more potent that *R*-enantiomer, but both enantiomers do not elicit different pharmacological effects (e.g. *S*-enantiomer is 20 times more potent in exerting negative dromotropic effect; 4 times more active in blood pressure reduction and equipotent with *R*-verapamil in the case of modulation of P170-mediated multidrug resistance)[49]. Antidepressant *S*-citalopram is also characterised by much higher potency than *R*-enantiomer in the inhibition of 5-hydroxytryptamine uptake. Therefore *S*-enantiomer, given the generic name escitalopram, has been marketed since 2002[38].

- Stereoisomers of which only one is active

Beta-receptor antagonists have only one active enantiomer. They contain at least one chiral centre and with the exception of timolol and penbutolol are administrated as racemates. The beta-blocking activity of *S*-enantiomer is at least two times higher for most beta-blockers than that of *R*-enantiomers[49].

3.3.2. Drugs with two enantiomers which are equally biologically active

This group includes only some drugs such as cyclophosphamide (antineoplastic) and fluoxetine (antidepressant)[36]. This group of enantiomers can be divided into two main subgroups:

- Stereoisomers that are equipotent

Enantiomers of antiarrhythmic drugs (flecainide, mexiletine, tocainide, propafenone) and antimalarials (mefloquine, halofantrine, enpiroline) have small or no differences in their potency[49].

- Stereoisomers that are both active but have qualitatively different actions

To this group belong chiral drugs with enantiomers being agonists at different receptors (e.g. dobutamine: (+)-enantiomer has β-blocking agonist activity, while (-)-enantiomer has α-blocking agonist activity), antagonists at different receptor (e.g. labetalol, beta-adrenoceptor antagonist having two chiral centres and four stereoisomers: *R,R*-enantiomer has β-blocking antagonist activity and *S,R*-enantiomers has α-blocking antagonist activity) and rarely agonists and antagonists at the same receptor (e.g. 1,4-dihydropyridines: one enantiomer behaves as calcium channel antagonist and the other one as calcium channel agonist)[49, 52]. Also enantiomers of α-propoxyphene differ in pharmacological actions. Whereas (+)-enantiomer is a potent analgesic, (-)-enantiomer is a potent antitussive agent[49, 53]. Some barbiturates also belong to this group. While *R*(-)-enantiomers are general anaesthetics, *S*(+)-enantiomers may be convulsant[48]. Also ketamine, an often abused anaesthetic, is more potent and less toxic in its *S*(+)-isomer form. *S*(+)-Ketamine is anaesthetic and analgesic, whereas *R*(-)-ketamine produces undesirable side effects such as hallucination and agitation[53]. The already mentioned thalidomide also belongs to this group. Other examples include: antiarthritic agent penicillamine (*S*-enantiomer has pharmacological action while *R*-enantiomer is extremely toxic) and the antitubercular agent ethambutol (*S,S*-enantiomer is an active tuberculostatic while *R,R*-enantiomer causes optical neuritis that can result in blindness). *L*-dopa,

used for treatment of Parkinson's disease, is marketed as single enantiomer because of the serious side-effects of *D*-isomer such as granulocytopenia[54].

3.3.3. Drugs with only one major bioactive enantiomer but with potential for the second non-active enantiomer to be transformed in body to its bioactive form through chiral inversion

Nonsteroidal anti-inflammatory drugs (ibuprofen, ketoprofen, etc) have an active *S*-enantiomer (e.g. *S*-ibuprofen is over 100 times more potent than *R*-ibuprofen). They can undergo enzyme mediated unidirectional inversion, which indicates that only an inactive *R*-enantiomer can undergo inversion into an active *S*-enantiomer. Benzodiazepines (*d*-enantiomer more potent than *l*-enantiomer) and thalidomide on the other hand undergo bidirectional chiral inversion or racemisation, which means that both *R* and *S* enantiomers can racemise in vitro by aqueous solution[36, 55, 56].

It has to be however emphasised here that different degrees of stereoselectivity can be observed for the same chiral drug regarding different effects as the stereoselective behaviour of chiral drugs is directly dependent on their modes of interaction with the macromolecules involved in eliciting certain pharmacological effects. For example, the decrease in heart rate mediated by beta-adrenoceptor antagonists is highly stereoselective for the *S*-enantiomer, while no enantioselectivity is observed for the local anaesthetic effects[49]. Furthermore, stereoselective disposition of drugs is also species dependant and as a result its understanding is of the greatest importance in understanding the environmental fate and ecotoxicity of chiral drugs.

3.4. Disposition of chiral drugs in humans

After administration a chiral drug is subject to a variety of physiological processes such as absorption, distribution, metabolism and excretion. Many of these processes are stereoselective as they involve an interaction between chiral drugs and chiral biological macromolecules[37, 38, 49].

3.4.1. Absorption, distribution and elimination

Drugs are usually absorbed by passive diffusion. As enantiomers do not differ in their physicochemical properties (lipophilicity, ionisation, molecular size), absorption is not usually considered to be a stereoselective process. However, stereoselectivity is expected and has been observed for drugs that are transported by a carrier-mediated process such as facilitated diffusion or active transport[37, 38, 49, 52, 57, 58]. Stereoselective transport of chiral drugs across the skin should also be mentioned here due to the possible effect it might have in terms of human exposure to chiral environmental contaminants. For chiral drugs with the biological activity associated with only one enantiomer, enantioselective permeation can for example affect the pharmacodynamic profile of the racemate. Although there is only limited information on the skin's stereoselective permeation, metabolism and binding, it has been established that stereoselectivity is observed in the case of some drugs (e.g. propranolol's transport through rat's skin)[59].

Furthermore, the distribution of chiral drugs in the body might be stereoselective as binding of chiral drugs to plasma or tissue proteins, and also transport via specific tissue, uptake and storage mechanisms can be stereoselective. As the drug in plasma that is not bound to proteins is responsible for pharmacological activity, differences in binding of enantiomers to proteins will affect their active concentration at the site of action. Competition between the pair of enantiomers for the same protein binding sites can also lead to higher free fractions of one enantiomer if the drug is administered in the racemic form. This might subsequently lead to changes in the disposition of chiral drugs when administered as racemate or single enantiomeric form. Furthermore, enantiomers of chiral metabolites can also stereoselectively bind to plasma proteins[38, 48, 49, 57, 58, 60].

The renal elimination of many chiral drugs by glomerular filtration and tubular secretion/reabsorption is stereoselective and can be affected by enantiomers' competitive stereoselective interactions with the anion/cation transport proteins in the renal tubular epithelial cells. These transporters have restricted capacity and as a result the enantiomers of racemic drugs

can compete for these sites potentially altering the disposition of each enantiomer when the racemate is given[49, 58, 60].

3.4.2. Metabolism

Stereoselective first-pass metabolism is observed in the case of many drugs and is considered to be rather a rule than an exception[38]. Among them are for example calcium channel blockers and beta-adrenoceptor antagonists e.g. active *S*-verapamil is more readily metabolised than *R*-enantiomer. Bioavailability of active *S*-propanolol is 1.5 times higher than its *R*-enantiomer also due to stereoselective first-pass metabolism[49].

Stereoselectivity of drugs metabolism can result from differences in the binding of chiral drugs to the enzyme active site and/or to catalytic sites. As a result two enantiomers of the same drug can be metabolised at different rates (by the same enzymes, e.g. verapamil) or via different routes (by different enzymes, e.g. warfarin and mephenytoin) and can lead to different products[38, 50]. There are several factors that might affect stereoselective metabolism. Among them are: disease, drugs interactions, ethnic differences, sex, age and lifestyle[52, 58, 60]. Significant consequences (inhibition or induction) of stereoselective metabolism might be observed in the case of two enantiomers, which differ in potency[49, 57]. Drugs interactions are also important. In the case when a pair of enantiomers is metabolised by different enzymes, a co-administered drug might inhibit the metabolism of one enantiomer only and not affect (or accelerate) the metabolism of the second enantiomer. If the metabolism of a more potent enantiomer is inhibited, this will obviously result in an increase of the drug's activity. On the other hand if inhibition of the not active enantiomer takes place, the opposite situation will be observed. Transport into bile and biliary elimination can be also stereoselective[49, 50, 61].

It should be also emphasised here that interspecies differences in the stereoselectivity of metabolism are very common. For example the metabolic oxidation of felodipine is greater for the *S*-enantiomer in humans, whereas a preferential metabolism of *R*-enantiomer takes place in rats and dogs[58]. Significant species differences exist in enantioselective pharmacokinetics for several drugs such as propranolol and warfarin where clearance shows the opposite stereoselectivity in animals compared to humans (propranolol: *S*>*R* in dog and *R*>*S* in humans; warfarin: *R*>*S* in rats and *S*>*R* in humans)[60]. The hydrolysis of esmolol also shows no stereoselectivity in human but in the case of dog and rat blood (-)-isomer is hydrolysed faster. On the other hand (+)-esmolol is hydrolysed faster in monkey, rat and guinea-pig blood[60]. Significant species difference is also observed in the case of chiral inversion of 2-aryl propionic acids[60].

Enantiomers of chiral drugs can be metabolised at different rates (leading to quantitative differences) or by different routes (leading to qualitative differences) resulting in preferential metabolism of one enantiomer as enzymes are chiral in nature[37, 49, 61]. In the case of warfarin both different rates and different routes are observed (Fig. 1).

Chiral drugs can be metabolised while retaining the same chiral centre in parent compound and metabolite, with an introduction of another chiral centre or the removal of chiral centre from the chiral molecule. In the first case, if the functional groups remain unaltered the absolute configuration of the parent compound and the metabolite will be the same, although the rate of biotransformation of each enantiomer can differ and the enantiomeric ratio of the chiral metabolite will also differ from that of the parent compound[49]. For example, *S*-warfarin undergoes aromatic oxidation with the formation of *S*-7-hydroxy-warfarin and *S*-6-hydroxywarfarin[38]. Such retention of stereoselectivity of the parent compound and metabolite is vital in the identification of the stereochemical form of the drug administered. This phenomenon is also utilised in forensic identification of illicitly used amphetamine where differentiation between legal and illicit usage of amphetamine can be estimated only through the analysis of enantiomeric ratios of excreted amphetamine. *S*-(+)-enantiomer of amphetamine is known to be much more potent than *R*-(-)-enantiomer and is present in illicitly used amphetamine either in pure or racemic form. On the other

hand *R*-(-)-enantiomer is often present in pharmaceutical preparations and/or excreted as a metabolite of certain drugs (e.g. selegiline) (Fig. 2).

Biotransformation of chiral drugs might also lead to the introduction of another chiral centre into a chiral molecule leading to the formation of diastereoisomers[49]. Among chiral drugs metabolised with the formation of additional chiral centre are: thioridazine (Fig. 3), metoprolol and bufuralol. The stereoselective glucuronidation of oxazepam, keto-reduction of warfarin also lead to the formation of diastereoisomeric derivatives[38, 62].

Metabolism can also lead to the removal of chiral centre from the chiral molecule. This process is characteristic for chiral calcium antagonists with dihydropyridine structure (e.g. nilvadipine) where oxidation of the dihydropyridine ring to the corresponding pyridine analogue leads to the loss of chirality. Oxidation of the benzimidazole proton pump inhibitors (e.g. omeprazole) at the chiral sulphoxide also leads to achiral sulphone (Fig. 4) [38, 49]. Deamination of amphetamine to yield phenylacetone also leads to the removal of chiral centre[63].

Some chiral drugs can undergo enzymatic chiral inversion, which results in the inversion of one enantiomer into the second one. This phenomenon is observed for non-steroidal anti-inflammatory 2-arylpropoinic acid derivatives such as: ibuprofen, benoxaprofen, cicloprofen, fenoprofen, flurbiprofen, ketoprofen and thioxaprofen, in which case chiral inversion of less potent *R*-enantiomer to the more potent *S*-enantiomers takes place. As a result of chiral inversion, despite revealing much higher potency of *S*-enantiomer *in vitro*, a significant decrease of such potency is observed *in vivo*. This is because not active *R*-enantiomer is a subject of inversion into more potent *S*-enantiomer. For example *S*-ibuprofen is 160 times more potent that *R*-ibuprofen *in vitro*, but only 1.4 times more potent *in vivo* (Fig. 5) [38, 48, 49, 56, 64]. Chiral inversion of NSAIDs takes place as a result of conjugation through a Coenzyme A (CoA) thioester intermediate. It is also worth emphasising that inversion by conjugation in other xenobiotic enantiomers can take place through other conjugation mechanisms such as glutathione. It has to be also noted here that metabolic chiral inversion of chiral drugs might be species dependant. For example *R*-flurbiprofen undergoes inversion in dogs and guinea pigs, but is negligible in rats and humans. Ketoprofen undergoes significant inversion in rats, dogs and horses (74-92%) with the smallest conversion in gerbils (27%) and humans (~10%). Microorganisms are also capable of chiral inversion. For example *Verticillum lecanii* inverts *R*-ibuprofen, fenoprofen and suprofen[56].

Finally, metabolic interactions between enantiomers of chiral drugs, leading to different disposition of chiral drugs when administered as racemate can be observed. The two enantiomers of the same drug can either compete for metabolism at the catalytic site of the same enzyme or one enantiomer can inhibit the enzyme for which the second enantiomer is a substrate. Such a phenomenon is observed in humans and animals in the case of several drugs such as: propafenone, nitredipine, propranolol, amphetamine, propoxyphene and warfarin[49]. The above has crucial implications in the decision making process of whether the distribution of chiral drugs as racemate or single enantiomer should be implemented.

It is also worth mentioning that metabolism of achiral drugs can lead to the formation of chiral metabolites through the introduction of a chiral centre[49, 63]. Among achiral drugs being metabolised to chiral metabolites are: haloperidol (antipsychotic), phenytoin (antiepileptic), debrisoquine (antihypertensive), cimetidine (H2-receptor antagonist), risperidone (antipsychotic) and some benzimidazoles (extensively used also in veterinary treatment) such as fenbendazole and albendazole[38, 48, 62, 63, 65]. Sulphide benzimidazoles are prochiral drugs and are used as anthelmintics. They are metabolised primarily to sulphoxides (active) and then to sulphones (not active). Sulphoxides are chiral and are responsible for most of the therapeutic activity (Fig. 6)[48, 66]. Sulphoxidation of cimetidine leads preferentially to the (+)-enantiomer of cimetidine sulphoxides[62]. Achiral phenytoin is metabolised to chiral 5-(4-hydroxyphenyl)-5-phenylhydantoin[67]. 9-Hydroxylation of risperidone and the metabolic formation of reduced haloperidol from haloperidol result in the formation of metabolites with chiral centres as presented in Fig. 6[65].

Due to stereoselective metabolism of chiral drugs and stereoselective formation of active intermediates and metabolites, the toxicity of enantiomers might also significantly differ[61]. For example the urotoxicity of racemic ifosfamide is caused by acrolein which is a breakthrough product of stereoselective dechloroethylation[49, 58]. Also in the case of male antifertility agents (3-chloropropane-1,2-diol and 3-amino-1-chloropropane-2-ol) studied on rats, only *S*-enantiomer revealed the antifertility activity while *R*-enantiomer is associated with nephrotoxicity due to its metabolism to *R*-3-chlorolactate leading to the formation of toxic 3-chloropyruvate[38]. The already mentioned *S*(-)-thalidomide is teratogenic as opposed to its sedative *R*(+)-enantiomer. Alitretinoin (9-cis-retinoic acid) and isotretinoin (13-cis-retinoic acid) are isomers of tretinoin all-trans retinoic acid. Their teratogenic effects are thought to be mediated as a result of their transformation to all-trans retinoic acid. Chiral structural analogues of valproic acid (VPA): 4-yn-VPA and 4-en-VPA are chiral and have varying teratogenic potential: *R*(+)-4-yn-VPA<*R*(+)-4-en-VPA<VPA<*S*(-)-4-en-VPA<*S*(-)-4-yn-VPA[35, 67].

The above discussion indicates that disposition of chiral drugs in the body is a very complex and often highly stereoselective process, which is potentially influenced by several parameters. Its understanding is therefore vital in informed decision making process concerning the administration of chiral drugs to humans and its possible consequences. It is also essential as far as environmental issues associated with the presence of chiral drugs in the environment are concerned, as chiral molecules are also subject to several complex and often stereoselective biological processes taking place in the environment, which might influence (often alter) the overall fate and ecotoxicity of these compounds. Unfortunately, only scarce information exists in the literature regarding the stereoselective fate and toxicity of chiral drugs in the environment. The following paragraph represents a short overview of current knowledge on chiral drugs in the environment.

4. Chiral drugs in the environment

4.1. Enantioselective drug analysis in environmental samples

Analysis of drugs at trace ppt concentrations in very complex environmental matrices poses a significant analytical challenge mainly due to problems associated with their separation from other interfering polar compounds. The above requires the application of sensitive analytical methods such as chromatography coupled with tandem mass spectrometry and combined with sample concentration/clean-up. Separation of enantiomers of drugs is even more problematic as traditional chromatography does not provide high enough selectivity to differentiate between enantiomers of the same compound. The development of robust methods for the separation of enantiomers of chiral compounds is therefore vital in the understanding of their occurrence and fate in the environment. Additionally, the establishment of multi-residue methods is crucial to obtain information regarding the cumulative presence of several groups of analytes at a particular place and time. This is of great importance as synergistic effects of different drugs on aquatic life might take place and have to be investigated. It has already been verified that acute toxicity of a group of pharmaceuticals can be higher than for individual pharmaceuticals. Although a few research groups have attempted to analyse single chiral drugs in environmental samples (Tab. 3), to date there are no multi-residue methods for the analysis of chiral drugs in environmental matrices.

On the other hand, extensive development of enantioselective analysis has taken place over the recent two decades in response to demand in drug development field for efficient technologies utilised in the preparation of enantiomerically pure compounds and quality control of these processes. Among the most popular separation techniques used for enantiomer analysis are liquid chromatography, followed by capillary electrophoresis and gas chromatography. Due to the limitations of space it is not possible to include full details on chiral separation techniques. The reader is therefore advised to seek more detailed information elsewhere. A few comprehensive books/book chapters[33, 34, 68-71] and reviews[51, 72-78] were published in this field in the recent few years.

Despite the availability of methods for chiral analysis of drugs in biological matrices it is difficult to directly utilise them in trace analysis of chiral drugs in the environment. This is because LC/UV is usually utilised in the chiral analysis of drugs in biological matrices and it is not sensitive and selective enough to be applied in environmental analysis, where usually tandem mass spectrometry has to be used. Direct transfer of chiral-LC/UV method to LC/MS/MS method is also not always possible because the first one usually uses mobile phases which are incompatible with MS applications (non-volatile buffers, normal-phase solvents). Additionally chiral drugs are usually of polar nature and therefore if chiral GC is to be used, a derivatisation step has to be undertaken before the analysis[31, 79, 80]. Therefore, although challenging, an extensive research in the field of chiral separation in complex environmental matrices is required in order to undertake research aiming at understanding the fate and toxicity of chiral drugs in the environment.

4.2. Occurrence, fate and toxicity of chiral drugs in the environment – current knowledge

4.2.1. Understanding environmental fate of chiral drugs

Chiral drugs are introduced into the environment as a result of human actions. Before they reach environmental matrices they are subjected to both biotic and abiotic processes (Fig. 7). Enantiomers of chiral drugs can be characterised by different biological fates in the environment because their interaction with chiral entities (e.g. enzymes, biological receptors) can be stereoselective[31]. On the other hand abiotic environmental processes (such as sorption, photochemical transformation, air-water, soil-air exchange) are not stereoselective because enantiomers of the same drug do not differ in physicochemical properties. Therefore stereoisomers can serve as markers of biological activity in the environment and can be an important source of information regarding biochemical fate of chiral compounds in the environment, which is required for accurate risk assessment of these pollutants[31, 79].

As a result of stereoselective biodegradation of chiral pollutants in the environment (and also during wastewater treatment) chiral products of varied toxicity can be formed. For example, γ-hexachlorocyclohexane is an achiral pesticide, but it degrades into chiral and toxic γ-pentachlorocyclohexene[33]. Enantioselective toxic effects are observed in the case of several chiral pollutants e.g. (+)-fipronil (phenylpyrazole pesticide) shows a significantly greater reduction in the number of off-spring of *Daphnia magna* than (-)-enantiomer[81]. Distribution of different enantiomers of the same pharmaceutical in the aquatic environment and biota can also be stereospecific. Therefore, the enantiomeric composition of chiral drugs can change significantly after its administration, followed by metabolism in and excretion from the human or animal body. It can be subsequently altered during biological wastewater treatment and as a result of biological degradation processes in the environment. Biological processes can lead to stereoselective enrichment or depletion of enantiomeric composition of chiral drugs. Therefore the very same drug might reveal different activity and toxicity and this will depend on its origin and exposure to several factors governing its fate in the environment.

Despite growing, but still limited, knowledge on the environmental fate of chiral pollutants such as agrochemicals (phenoxyalkanoic acid herbicides, acetamide and organophosphorous and pyrethroid insecticides), polychlorinated biphenyls and their metabolites, and synthetic polycyclic musks (see reviews by Wong[31], Müller and Kohler[32], Hühnerfuss and Shah[81]) no comprehensive research has been undertaken in the field of chiral drugs. Existing reports on the presence and fate of pharmaceuticals, due to their non-enantiospecific analysis, do not tackle the problem of their chirality, so these studies cannot differentiate between different biological (enantioselective: microbial transformation processes, enzymatic transformation) and abiotic (non-enantioselective: photochemical transformation) transformation processes to which chiral drugs are exposed. As a result, current knowledge of chiral pollutants, especially drugs, is inaccurate, as it incorrectly assumes that enantiomers have identical environmental behaviour[31].

Therefore to understand and predict the mechanisms governing the fate of chiral drugs, their possible toxicity and impact on the environment, determination of their enantiomeric composition

in environmental matrices is essential. As only a few reports exist on the analysis of chiral pharmaceuticals (beta-blockers, no metabolites) in the environment[82, 83] and during wastewater treatment[79, 84-88], it is of the greatest importance to investigate this aspect of the presence of drugs in the environment.

The relative concentration of enantiomers of chiral drugs can be expressed as the enantiomeric fraction (*EF*)[83]:

$$EF = [enantiomer\ 1]/([enantiomer\ 1] + [enantiomer\ 2])$$

EF equals 1 or 0 in the case of single enantiomer form and 0.5 in the case of racemate. Changes in *EF* for the same chiral drugs can be therefore used to identify enantioselective processes.

Fono and Sedlak[83] reported that propranolol was racemic in the influent of studied WWTPs (*EF* = 0.49 - 0.54) but not in the effluent wastewater (*EF* = 0.31 - 0.44) after activated sludge treatment (Tab. 4). The highest *EF* values were observed for WWTP characterised by poor removal during secondary biological treatment and during wet weather conditions when 9% of the effluent sample consisted of raw sewage that bypassed secondary treatment. It is worth noting here that *EF* values were found to decrease after biological treatment but remained constant after chemical and physical treatment (filtration, settling and chlorination), which suggests stereoselective processes occurring during biological treatment. Fono et al.[82] also studied the fate of metoprolol in river. A decrease of *EF* values for metoprolol alongside the river (from *EF*=0.5 to *EF*=0.44 over travel time=13 days) indicated its biotransformation (Tab. 5).

Nikolai et al.[79] studied enantioselective degradation of three *β*-blockers: atenolol, metoprolol and propranolol during wastewater treatment. It was reported that all compounds studied were a subject to enantioselective biodegradation (Tab. 4). It was also concluded that this process is season-dependant and possibly results from changes in populations and selectivity of microbes capable of degrading the analyte. Additionally different stereoselectivity was observed in different WWTPs suggesting possible different enantioselectivity of biochemical weathering processes[79].

MacLeod et al.[84] studied β-blockers (atenolol, metoprolol, propranolol, pindolol, nadolol and sotalol), SSRI (citalopram and fluoxetine) and salbutamol during wastewater treatment and also observed changes in *EFs* of several drugs as a result of wastewater treatment (Tab. 4). Wastewater influent was slightly enriched with *R*(+)-atenolol, while the effluent was racemic. The influent was also more enriched with *R*(-)-fluoxetine than effluent[84]. Unfortunately no studies were undertaken for the main active chiral metabolite *S*(-)-norfluoxetine. An enrichment of fluoxetine with *S*(-)-enantiomer as a results of wastewater treatment is of potentially significant toxicological consequences as toxic effects of fluoxetine enantiomers are species dependent: *S*-fluoxetine is more toxic than *R*-fluoxetine in *Pimephales promelas*, but equal toxicity of both enantiomers in the case of *Daphnia magna* is observed[89]. Propranolol on the other hand was found to be racemic in wastewater influent. Effluent in contrast was enriched with *S*(-)-propranolol, which is known to have higher toxicity towards *Pimephales promelas* than its antipode[84, 89]. MacLeod et al.[87] undertook also a several month long monitoring programme of chiral drugs (atenolol, citalopram, fluoxetine, metoprolol, nadolol, pindolol, propranolol, salbutamol, sotalol and temazepam) in WWTPs' effluents. With the exception of temazepam, chiral drugs were generally non-racemic. Temporal changes in *EFs* values of studied chiral drugs (with the exception of sotalol) were observed and some differences in *EFs* were also noted among studied WWTPs. It was suggested that there might be some variation of microbial transformation of drugs in WWTPs among plants and treatment processes. It was however pointed out that temporal differences in enantiomer-specific metabolism of humans might also contribute to the overall temporal variation in chiral drug *EFs* observed in WWTPs effluents[87].

Matamoros et al.[85] reported that enantioselective degradation of ibuprofen depends on the oxidation status of WWTP. In predominantly aerobic conditions *S*-ibuprofen degrades faster than *R*-ibuprofen (Tab. 4). On the other hand in anaerobic conditions degradation of ibuprofen is not enantioselective.

It is suggested that such a situation is a result of the presence of different bacterial consortia under aerobic and anaerobic conditions. In contrast *EF* of naproxen decreased during wastewater treatment in the case of both aerobic and anaerobic processes[85]. Different enzymatic metabolisms of ibuprofen and formation of the metabolites: hydroxyibuprofen and carboxyibuprofen, encountered in the human body, in a sewage treatment plant and in rivers were also observed and reported by Hühnerfuss and Shah[81]. Buser et al.[90] also studied the occurrence and behaviour of ibuprofen during wastewater treatment and in surface water. Ibuprofen was found at very high concentrations in WWTP influents with a high enantiomeric excess of the pharmacologically active *S*-enantiomer, which significantly decreased as a result of wastewater treatment (Tab. 4). In rivers and lakes, ibuprofen was found at much lower concentrations with some excess of the pharmacologically active *S*-enantiomer[90] (Tab. 5). Winkler et al.[91] studied ibuprofen in a biofilm reactor with river water and observed much higher degradation of non-pharmacologically active *R*-enantiomer. This indicates that the principlal environmental contaminant resulting from the use of ibuprofen is *S*-enantiomer, which is pharmacologically active to humans and probably to other vertebrates and possibly invertebrates.

Kasprzyk-Hordern et al.[88] studied several drugs of abuse such as amphetamines (amphetamine, methamphetamine, MDEA, MDMA and MDA), ephedrines (ephedrine, pseudoephedrine and norephedrine) and venlafaxine during wastewater treatment. The study of enantiomeric fractions of these chiral drugs proved their non-racemic composition (Tab. 4). It was for example observed that in the case of methamphetamine, only the more potent *S*(+)-enantiomer was detected in all treated wastewater samples. The opposite situation was observed in the case of amphetamine, where less potent *R*(-)-enantiomer was present in both raw and treated wastewater at slightly higher concentrations than *S*(+)-enantiomer. The study of enantiomeric fractions of MDMA indicated the predominance of enantiomer 1 in both raw and treated wastewater samples. However, in the case of initially racemic venlafaxine enrichment of this drug with *E2*-enantiomer was observed as a result of wastewater treatment. This again might suggest enantioselective processes occurring.

In summary, very limited research on enantioselective fate of chiral drugs in the environment has been undertaken so far. The available results clearly indicate that enantioselective processes occur both during wastewater treatment and in the environment, although more comprehensive research has to be undertaken to fully support such a hypothesis. The main difficulty is associated with the prediction of stereoselective pathways of chiral pollutants in the environment as this process is dependent on the environmental system, the species and the organ. Racemisation and enantiomerisation processes can also occur and make interpretation of the data even more complex[32]. Enantioselective metabolism patterns in humans and animals to which chiral drugs were administered before their excretion into the environment have to be also considered.

4.2.2. Enantioselective toxicity of chiral drugs in the environment

Minimal data only exists on enantioselective toxicity of chiral drugs in the environment. Stanley et al.[89] studied enantiospecific effects of fluoxetine on *Pimephales promelas* (teleost) and *Daphnia magna* (crustacean). *S*-fluoxetine was found to be more toxic in *P. promelas*, potentially because its primary active metabolite, *S*-norfluoxetine is more potent than *R*-fluoxetine in mammals. This was not observed for *D. magna* responses, where both enantiomers revealed similar toxic effects[89]. The same group studied enantiospecific toxicity of propranolol[93]. Acute 48h responses of *P. promelas* and *D. magna* were similar for both enantiomers. Chronic *P. promelas* responses to propranolol revealed higher chronic toxicity of *S*-propranolol, but chronic *D. magna* did not follow this pattern[93].

Lack of toxicological data referring to possible enantioselectivity in toxic responses of aquatic organisms in the presence of chiral drugs is surprising and also disturbing. Currently, toxicity of chiral drugs towards aquatic organisms is commonly assessed for racemic forms of drugs only (or without any specification of which form should be used) and therefore is inaccurate, as it incorrectly assumes that enantiomers have identical toxicity. Therefore extensive research is needed here to

verify existing ecotoxicological data in the context of chirality of pharmacologically active compounds.

4.3. Chiral drugs of environmental concern

Below only the most important environmentally relevant groups of chiral drugs will be discussed due to constraints regarding manuscript length. Among them are: NSAIDs, analgesics, anaesthetics, CNS drugs, cardiovascular drugs, respiratory drugs, gastro-intestinal drugs, antimicrobials and chemotherapy drugs.

4.3.1. NSAIDs, analgesics and anaesthetics

4.3.1.1. NSAIDs

NSAIDs are the most widely studied chiral drugs in humans in terms of their stereoselective action. Chiral NSAIDs are used as anti-inflammatory, antipyretic and analgesic agents. Among 2-arylpropionic acids (2-APA) there are: ibuprofen, ketoprofen, fenprofen, flurbiprofen, tiaprofenic acid, carprofen, pirprofen, benoxaprofen and naproxen (Fig. 8). Non-APA chiral NSAIDs include: etodolac and ketorolac both marketed as racemates[67, 57] (Fig. 8). Chiral NSAIDs are marketed mainly as racemic mixtures although the following NSAIDs: naproxen (marketed only as *S*-naproxen), dexibuprofen (chiral switch of ibuprofen) and dexketoprofen (chiral switch of ketoprofen) are also distributed as single enantiomers (Tab. 6).

Several NSAIDs are present in over-the-counter medications and are also prescribed in high quantities all over the world. For example ibuprofen is one of the top-ten drugs sold worldwide[94]. Annual consumption of ibuprofen in Germany accounted for 345 tonnes in 2001[2]. Annual prescription data in England for several chiral NSAIDs is presented in Tab. 6. Racemic ibuprofen and *S*-naproxen were prescribed in 2007 in >100 and >30 tonnes respectively. Ibuprofen is also prescribed as single *S*-enantiomer but at much lower quantities: 0.5 tonne year^{-1} [19]. Ketoprofen is also marketed as both racemate and *S*-enantiomer. Similarly to ibuprofen, its prescription in England as one active enantiomer is much lower than racemate.

Pharmacodynamics and pharmacokinetics

NSAIDs act by inhibiting the two isoforms of cyclooxygenase enzyme (COX-1 and COX-2), which catalyse the synthesis of different prostaglandins from arachidonic acid. Prostaglandins are involved in processes such as inflammation and pain, regulation of blood flow in kidney, coagulation processes and synthesis of protective gastric mucosa. Because NSAIDs non-specifically inhibit prostaglandin synthesis, most side effects are related to physiological functions of prostaglandins and might involve renal and gastric damage. Prostaglandins are also formed in many vertebrates and invertebrates; however in lower invertebrates their synthesis involves usage of different enzyme. In birds prostaglandins play a role in the biosynthesis of egg shells and therefore COX-inhibitors (such as indomethacine) can lead to shell thinning[2, 94].

Most chiral NSAIDs are extensively metabolised in humans and are excreted mainly as conjugates. Metabolic conjugation of drugs with polar molecules such as glucuronic acid is common and should be taken into consideration when assessing environmental exposure to these drugs. Unfortunately it is rarely reported. In the case of ibuprofen, less than 10% of the administered dose is excreted unchanged, 9% of the dose accounts for the 2-hydroxy metabolite (2-[4-(2-hydroxy-2-methylpropyl)phenyl]propionic acid), 17% accounts for the conjugated 2-hydroxyibuprofen, about 16% is excreted as the 2-carboxy metabolite (2-[4-(2-carboxypropyl)phenyl]propionic acid) and about 19% as the conjugated carboxyibuprofen[96]. Two minor metabolites are also formed: 1- and 3-hydroxyibuprofen. Both hydroxy and carboxyibuprofen are chiral. However, only in the case of carboxyibuprofen and 1- and 3-hydroxyibuprofen the introduction of a second chiral centre in the molecule is observed (Fig. 9). Naproxen is excreted mainly as conjugated naproxen (60% of the dose), 6-*O*-desmethylnaproxen (5%) and conjugated desmethylnaproxen (20%). Less than 10% of

the excreted material is unchanged drug. Ketoprofen on the other hand is excreted as glucuronide conjugate (90%); hydroxylation may also occur[96].

Some 2-APA derivatives undergo a unidirectional chiral inversion from the inactive *R*- to the active *S*-enantiomer (e.g. ibuprofen, ketoprofen and fenoprofen). Chiral NSAIDs are also subject to drug-dependent stereoselectivity in microsomal oxidation and/or glucuronidation processes. The clearances of ibuprofen metabolites are higher for *S*- than *R*-enantiomer. Renal clearance of *S*-ketoprofen glucuronide is also higher than that of the *R*-enantiomer. On the other hand, renal clearance of tiaprofenic acid and flurbiprofen is not stereoselective and in the case of both drugs chiral inversion is very likely to occur. Etodolac shows stereoselectivity in clearance of the enantiomers through glucuronidation, with *S*-enantiomer having 13 times higher value than antipode. Ketorolac does not undergo chiral inversion[58, 67, 97].

2-APA derivatives such as ketoprofen, ibuprofen, naproxen, carprofen, vedaprofen, oxindanac, flurbiprofen, fenoprofen are also used in veterinary treatment. Similarly to humans, chiral inversion and enantioselective disposition in animals is characteristic for this group of chiral drugs. However, it has to be emphasised here that the extent of both processes varies between different species[48, 98]. For example fenoprofen has a chiral inversion rate of 90% in dogs, 80% in sheep, 73% in rabbits, 60% in man, 42% in rats and 38% in horses[48]. Similarly ketoprofen reveals different chiral inversion rate in different species: 6% in male sheep (Corriedale), 9% in man, 14% in female sheep (Dorset Cross), 15% in goat, 22% in cat, 31.7% in calf and 49% in horse[99]. Tiaprofenic acid also shows little chiral inversion in humans but is significant in rats[58]. The phenomenon of metabolic inversion has not only pharmacological consequences but also toxicological consequences such as formation of hybrid triglycerides and even inhibition of fatty acid β-oxidation[100].

The above discussion indicates that the phenomenon of stereoselectivity of NSAIDs transformation is very complex and not uniform for all species, which might significantly complicate research efforts aiming to understand the stereoselectivity of these drugs in the environment, their fate and toxicity. On the other hand, the above commentary shows that enantioselectivity of NSAIDs is crucial in understanding their action and transformation in biological systems. Therefore studies on chirality of NSAIDs have to be considered as a vital dimension in environmental research aiming to understand their fate in the environment.

Environmental occurrence and toxicity

Due to NSAIDs' high worldwide distribution as human and veterinary pharmaceuticals, their widespread occurrence in the environment is to be expected. NSAIDs have an acidic nature and pKa values varying from 3 to 5, therefore at neutral conditions they are present in ionised form in the environment. As a result NSAIDs have very little tendency to adsorb to sludge and sediments but adsorption increases with lower pH. Biodegradation of NSAIDs is believed to be the most important factor leading to the removal of these compounds during wastewater treatment. Both aerobic and anaerobic processes can take place. The efficiency of their removal during wastewater treatment is compound and WWTP dependant and can vary from 0 to 100%[2, 101]. For example in Canadian studies of 12 WWTPs, ibuprofen and naproxen were removed with high median reduction greater than 93%. Ketoprofen on the other hand was characterised by lower removal at a median of 44%[102]. Similar results were observed by other research groups[103-107]. Santos et al.[107] observed varying efficiency of ketoprofen and naproxen removal in several WWTPs in Spain accounting for 38-67% and 40-90% respectively. Ibuprofen removal rates were very high, 88-93%[107]. On the other hand Castiglioni et al.[108] observed season dependant removal efficiencies of ibuprofen accounting for 0 to 100% (lower in winter, higher in summer). Kasprzyk-Hordern et al.[9] reported varying removal efficiencies of NSAIDs due to the utilisation of different wastewater treatment processes: activated sludge and trickling filters. Activated sludge resulted in a much higher removal efficiency of ibuprofen, naproxen and ketoprofen (92, 78 and 74% respectively) than trickling filters (85%, 58% and 57% respectively)[9].

Biodegradation of NSAIDs also occurs once they are present in the environment but also abiotic degradation such as photodegradation (observed for example in the case of naproxen and ketoprofen) can take place[109]. NSAIDs are frequently detected at high concentrations in the μg L^{-1} range in treated wastewater and, as a result of insufficient wastewater treatment, at ng L^{-1}, reaching at times μg L^{-1} levels in surface waters (Fig. 10). NSAIDs such as ibuprofen, ketoprofen and naproxen were also quantified in drinking water in France and Finland. Their maximum concentrations in drinking water were as follows: 0.6 and 8.5 in the case of ibuprofen, 3 and 8 in the case of ketoprofen and 0.2 ng L^{-1} in the case of naproxen[110, 111]. Loraine and Pettigrove[112] quantified ibuprofen in finished drinking water at concentrations ranging from 0.51 to 1.35 μg L^{-1}. It has to be however emphasised here that reported levels of NSAIDs in environmental matrices might be inaccurate as conjugated or transformation forms of studied drugs are rarely taken into consideration. Despite the frequent occurrence of NSAIDs in the environment and significantly different pharmacological activity of the enantiomers of chiral NSAIDs, only limited research has been undertaken on the enantioselective fate of NSAIDs in the environment and is discussed in paragraph 4.2.1. Results of studies undertaken by Buser et al.[90], Winkler et al.[91] and Matamoros et al.[85] show that while active *S*-ibuprofen is more readily metabolised in humans an opposite situation is observed in environmental samples. This indicates that the principal environmental contaminant resulting from the use of ibuprofen is pharmacologically active to humans and probably to other vertebrates and possibly invertebrates *S*-ibuprofen[91]. Lack of research regarding the enantioselective fate of NSAIDs is surprising especially because rather comprehensive knowledge exists concerning environmental enantioselective processes for their structural analogues – phenoxyalkanoic acid herbicides such as mecoprop and dichlorprop[31].

Several studies have been also undertaken for metabolites of NSAIDs but again no enantioselective research took place despite the fact that several NSAIDs' metabolites are chiral. Ibuprofen and its metabolites have been studied (without taking into consideration the phenomenon of chirality) by several research groups[91, 113-115]. It was observed that microbial biodegradation of ibuprofen leads to the formation of the same metabolites as human metabolism. These are: carboxy- and hydroxyibuprofen, both chiral molecules. Laboratory studies indicated that both metabolites degrade in a river biofilm reactor. However, in human metabolism the metabolite carboxyibuprofen appears and degrades second whereas the opposite occurs in biofilm systems[91]. Batt et al.[116] quantified 2-hydroxyibuprofen at higher levels than ibuprofen (88-72 ng L^{-1} and 67-200 ng L^{-1} of ibuprofen and 2-hydroxyibuprofen respectively) in wastewater effluent samples. Weigel et al.[114] observed predominant occurrence of hydroxyibuprofen in WWTP effluents and rivers whereas carboxyibuprofen was dominant in seawater samples, which suggests different transformation behaviour under freshwater and marine conditions. The above discussion indicates that the formation of metabolites is of great importance in understanding the fate of NSAIDs in the environment and during wastewater treatment. More importantly, understanding of enantioselective behaviour of chiral NSAIDs and formation of their chiral metabolites in the environment is of crucial importance in comprehensive and accurate verification of their fate and ecotoxicity, as metabolites might reveal equal or even higher toxic effects to their parent molecules.

NSAIDs were found to have relatively low acute toxicities (EC_{50} > 100mg L^{-1})[94], although they were found to vary for different NSAIDs and organisms studied. For example acute toxicity of naproxen in different organisms vary from 12.3 mg L^{-1} (*Cyanobacteria*) to 690 mg L^{-1} (*O. mykiss*)[117]. Acute toxicity of ibuprofen is 9.1, 7.1 and 173 mg L^{-1} in the case of daphnid (48h), algae (24h) and fish (<96h) respectively. Acute toxicity of naproxen was found to be lower and indicated 37, 21 and 560 mg L^{-1} in the case of daphnid (48h), algae (24h) and fish (<96h) respectively. In the case of ketoprofen acute toxicity for daphnid (48h) was found to be 64 mg L^{-1} [10]. The above discussion indicates that NSAIDs are toxic (EC_{50} = 1-10 mg L^{-1}) or harmful (EC_{50} = 10-100 mg L^{-1}) to crustaceans and harmful to fish[3]. Synergistic effects of several NSAIDs and their effect on toxicity were also verified. Toxicity of the mixture of a few NSAIDs was found at concentrations where single pharmaceuticals showed no or only minimal effects[2]. Higher toxicity of naproxen photodegradation by-products to rotifer *Brachionus calyciflorus*, the water flea *Ceriodaphnia dubia*

and the fairy shrimp *Thamnocephalus platyurus* than parent compound was also reported[212]. As acute toxicity tests do not take into consideration actual long-term environmental exposure to NSAIDs at reported environmental levels, their actual potential hazard cannot be underestimated. For example chronic toxicity studies indicated that ibuprofen at environmentally relevant concentrations (1 and 10 ng/L) is responsible for an activity decrease of freshwater amphipod *Gammarus pulex*[212]. In the case of photosynthetic organisms a five day exposure to ibuprofen at concentrations ranging from 1 to 1000 µg/L stimulated the growth of the cyanobacterium *Synechocystis* but inhibited, after seven days, growth of the duckweed plant *Lemna minor*[212].

Unfortunately no research on stereoselective toxicity of NSAIDs has been undertaken to date, despite the fact that enantiomers of NSAIDs significantly differ in their activity. As enantiomeric composition of chiral drugs is not taken into consideration, ecotoxicity data obtained for racemic drugs might lead to under- or overestimation of overall toxicity of the chiral compound and as a result indicate the high inaccuracy of currently available toxicological data. This could be the case if ibuprofen is taken into consideration. It is observed to be present in the environment with an excess of pharmacologically active *S*-enantiomer, which might reveal higher toxicity towards certain organisms than racemic ibuprofen.

4.3.1.2. Analgesics

Among chiral analgesics are: morphine, methadone, propoxyphene, tramadol, medetomidine, nefopam, eletriptan, zomitriptan, hydromorphone, pentazocine, methylsergide and several others (Fig. 8). All reveal enantioselective pharmacokinetics. Naturally occurring opiates (e.g. codeine and diamorphine) are distributed in the form of (-)-enantiomer, while synthetic opiates such as tramadol are marketed as racemates (Tab. 6).

Analgesics are distributed worldwide in very high quantities. For example in England only, annual prescription of tramadol, codeine and dihydrocodeine in 2008 accounted for 30, 38 and 11 tonnes respectively (Tab. 6). Additionally, the usage of analgesics in England reveals a steadily growing trend (Tab. 6).

Pharmacodynamics and pharmacokinetics

Analgesics are readily metabolised and excreted in the form of conjugates (glucuronide or sulphate) or as more polar metabolites. In the case of codeine, 40 to 70% of excreted material accounts for free or conjugated codeine, 5-15% free or conjugated morphine, 10-20% free or conjugated normorphine. Metabolism of dihydrocodeine includes *N*-demethylation to form nordihydrocodeine, *O*-demethylation producing dihydromorphine, 6-keto reduction, and conjugation. Diamorphine, following injection, is rapidly metabolised to 6-monoacetylmorphine and then more slowly metabolised to morphine, which is the major active metabolite. Orally administered diamorphine undergoes extensive first-pass metabolism to morphine and is excreted mainly in the form of morphine-3-glucuronide and 5-7% of the dose as free morphine, 1% as 6-acetylmorphine[96].

Morphine and codeine are used as naturally occurring single (-)-enantiomers with 5 chiral centres[118]. The opiate receptors are stereoselective and pharmacological activity is dependant on configuration. For example synthetic (+)-morphine has very weak affinity for opiate receptors. Metabolism of (+) and (-)-morphine is also stereoselective. 3-*O*-glucuronide is preferred in the case of (-)-morphine, while 6-*O*-glucuronide is preferred in the case of (+)-enantiomer.

Tramadol has two chiral centres and as a result four stereoisomers. Clinically it is used as a mixture of two enantiomers: *1R,2R*(+)-tramadol and *1S,2S*(-)-tramadol[57]. (+)-Tramadol and also (+)-enantiomer of its active *O*-demethylated metabolite show higher analgesic potency than (-)-enantiomer[67]. Tramadol is extensively metabolised. The main metabolic reactions are *N*- and *O*-demethylation and conjugation with glucuronic acid and sulphate. The major metabolites formed are: *O*-monodesmethyltramadol, *N,O*-didesmethyltramadol and their conjugates, and *N*-desmethyltramadol. About 30% of a dose is excreted unchanged[96].

Methadone has one chiral centre and is commonly used as racemate[118]. It is metabolised with the formation of non-active major 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidone (EDDP, 43% of the dose) and 2-ethyl-5-methyl-3,3-diphenyl-1-pyrrolidone (EMDP, 5-10% of the dose). About 33% of the dose is excreted unchanged[96]. *R*(-)-enantiomer of methadone is therapeutically active[119]. It shows 10 times higher affinity towards μ and κ-receptors and up to 50 times the antinociceptive activity than *S*-enantiomer. Moreover, only *R*-enantiomer prevents opioid withdrawal syndrome. Both enantiomers reveal significant differences in pharmacokinetics. *R*-methadone shows a significantly higher unbound fraction and total renal clearance than *S*-methadone. Furthermore, plasma clearance of the fraction that was not protein bound is significantly lower for *R*-methadone[41, 50, 119].

Propoxyphene is used as a single enantiomer - dextropropoxyphene[118]. In the case of nefopam, which is used as racemate, (+)-enantiomer is 7-30 times more potent than (-)-enantiomer in 5-HT, noradrenaline and dopamine binding sites[119]. Medetomidine is widely used in veterinary anaesthesia. Anaesthetic potency lies mainly in its *D*-enantiomer - dexmedetomidine[57].

Environmental occurrence and toxicity

Similarly to NSAIDs, some analgesics are commonly quantified in the aqueous environment at levels reaching 100 ng L^{-1} in surface water (Fig. 10) although their occurrence and fate were not as extensively studied. Codeine is the most widely studied and found in surface water at high levels reaching μg L^{-1} (Fig. 10). This is due to its high usage and low to moderate removal during wastewater treatment. Codeine was found to be removed during WWTP treatment with moderate efficiency accounting for 46%±19%[103]. An average of 37±36% and 42±30% removal of codeine was observed in the case of trickling filters and activated sludge treatment respectively[9]. Wick et al.[120] reported >80% removal of codeine and morphine as a result of activated sludge treatment. However, only limited removal of other opioids (dihydrocodeine, methadone and tramadol) was observed[120].

Despite the fact that analgesics are readily metabolised and excreted in the form of several metabolites, their fate, when taking into account active metabolites was hardly taken into consideration. Also, no stereoselective occurrence and fate of these compounds have been reported so far. No or very limited environmental toxicity studies have been carried out for this group of compounds. Acute toxicity of tramadol was found to be 73 mg L^{-1} for daphnid (48h) and 130 mg L^{-1} for fish (<96h)[10], which indicates that this compound is harmful to crustaceans but not toxic to fish. Unfortunately, no relevant stereoselective toxicity studies were undertaken.

4.3.1.3. Anaesthetics

Several anaesthetics are chiral (approximately 60%) and many are used as racemates. Among general anaesthetics are: ketamine, thiopental and also racemic fluorinated agents administered by inhalation: halothane, enflurane, isoflurane and desflurane (Fig. 8). Chiral local anaesthetics include: bupivacaine, mepivacaine and prilocaine[57, 67].

Pharmacodynamics and pharmacokinetics

Anaesthetics similarly to analgesics are readily metabolised and excreted in the form of conjugates or as more polar metabolites. Thiopental is a barbiturate and is widely used in anaesthesia. *S*-thiopental is known to be more potent and has a lower safety threshold than *R*-thiopental. Chiral pentobarbital, one of the major metabolites of thiopental, also reveals anaesthetic potency[57, 67]. Chiral etomidate is used as *R*(+)-enantiomer in clinical practice as this enantiomer reveals five times higher anaesthetic potency than *S*(-)-enantiomer[57]. Ketamine is distributed as a racemate (or as *S*(+)-enantiomer in some countries). It reveals stereoselectivity in both pharmacological and clinical effects. *S*(+)-enantiomer reveals higher hypnotic and analgesic potency than *R*(-)-ketamine[38, 57]. *R*(-)-ketamine on the other hand is responsible for side effects in surgical patients such as: hallucinations, restlessness and agitation[50]. Ketamine in vivo is demethylated to

norketamine, which retains the chiral centre. It is also used in veterinary treatment. Similarly to humans, *S*(+)-ketamine is approximately 3 times more potent than *R*(-)-ketamine in rats and mice[48].

Local anaesthetics prilocaine, mepivacaine and bupivacaine reveal stereoselective pharmacokinetics[50]. Both mepivacaine and prilocaine are distributed as racemates. Their enantiomers have similar local anaesthetic potency, but they differ in several other aspects resulting in *S*(+)-enantiomers having longer duration of action[121]. *S*(+)-bupivacaine was also found to have similar anaesthetic potency as *R*(-)-bupivacaine but the latter was found to be more toxic (causing cardiac arrhythmias)[67, 122]. Animal toxicity studies have revealed a 50% higher systematic toxicity for R-enantiomer attributable to cardiotoxicity and CNS toxicity[123]. Therefore bupivacaine is distributed as racemate and also as a single *S*(-)-enantiomer (levobupivacaine).

Hyoscine used in anaesthesia, just like other naturally occurring drugs (e.g. morphine, adrenaline, noradrenaline and tubocurarine) is synthesised and administered as single stereoisomer. Atropine (the racemic form of hyoscyamine) is an exception and is distributed as racemate. Atropine is present in plants as an *l*-isomer but it is converted to a racemic mixture during extraction. As *d*-isomer has little or no anticholinergic activity, the overall potency of racemic atropine is reduced in 50%[121].

Environmental occurrence and toxicity

Limited or no data exists on the occurrence of anaesthetics in the environment and their ecotoxicity. No stereoselective studies were undertaken for this group of compounds. Ketamine was the only compound studied in environmental matrices and quantified at low ng L^{-1} levels in wastewater effluent[6].

4.3.2. CNS drugs - psychiatric drugs

An understanding of the role chirality plays in pharmacology is of crucial importance in psychiatry, where the majority of commonly used antidepressants, antipsychotics and benzodiazepines are chiral and introduced as racemates. Enantiomers very often reveal different pharmacological effects and side effects.

4.3.2.1. Antipsychotic drugs

Structures of chiral antipsychotic drugs are presented in Fig. 11. Among them are: thioridazine, sulpiride and methotrimeprazine.

Pharmacodynamics and pharmacokinetics

Thioridazine is a chiral antipsychotic drug sold as racemic mixture of two enantiomers. Thioridazine contains one chiral carbon and is administered as racemate. It is metabolised to two sulfoxidated metabolites: 2-sulfoxide thioridazine (mesoridazine) and 5-sulfoxide thioridazine (Fig. 3), in which a second chiral centre in the form of mono-oxidised sulphur atoms is incorporated. Mesoridazine is metabolised further to sulforidazine[67]. Both mesoridazine and sulforidazine are pharmacologically active and have been launched as antipsychotics in some countries. Thioridazine-2,5-disulfoxide contains three chiral centres. Many of the thioridazine stereoisomeric metabolites contribute to the racemate's pharmacodynamics and cardiovascular toxicity (e.g. thioridazine 5-sulfoxide is a cardio-toxic metabolite with four enantiomers)[41].

Methotrimeprazine is marketed as a racemate. (-)-Enantiomer reveals higher affinity towards dopamine receptors[65]. Sulpiride on the other hand is distributed in some countries as a racemate or *S*-sulpiride in others. *S*-sulpiride is a more potent antagonist at dopamine D2 receptors than *R*-enantiomer[65]. Sulpiride is not metabolised to a great extent[96]. Prochiral risperidone is metabolised in liver with the formation of active (equipotent with parent drug) racemic chiral 9-hydroxyrisperidone[124].

Environmental occurrence and toxicity

Limited data can be found in literature on the occurrence and ecotoxicity of antipsychotic drugs. Sulpiride was quantified at high concentrations exceeding 100 ng L^{-1} in wastewater in China[125]. Low removal efficiencies (<40%) of this compound were also observed during conventional biological wastewater treatment. However, the application of advanced treatment involving ozonation or microfiltration/reverse osmosis resulted in >90% removal of this compound[125]. Acute toxicity of thioridazine was found to be high and denoted 4.56 and 0.33 mg L^{-1} in the case of daphnid (48h) and fish (<96h) respectively, which indicates that this compound is very toxic to fish (EC_{50} = <1 mg L^{-1}) and toxic to crustaceans (EC_{50} = 1-10 mg L^{-1}). In the case of risperidone acute toxicity for daphnid (48h) was found to be also high: 6 mg L^{-1} [10]. Unfortunately, despite common usage, no stereoselective studies were undertaken for this group of racemic drugs in the environment.

4.3.2.2. Antidepressants

Among chiral antidepressants (Fig. 11) are selective serotonin reuptake inhibitors (SSRIs: fluoxetine, citalopram, paroxetine, sertraline), serotonin-norepinephrine reuptake inhibitors (venlafaxine), norepinephrine reuptake inhibitors (reboxetine), tricyclic antidepressants (trimipramine), tetracyclic antidepressants (mirtazapine, mianserin), monoamine oxidase inhibitors (tranylcypromine), and several other drugs. Fluoxetine is a commonly prescribed racemic antidepressant and is one of the best-selling drugs in the USA. In England its annual prescription exceeds 3 tonnes and is steadily growing (Tab. 7). Similarly the annual prescription quantity of citalopram (>3 tonnes), sertraline (>4 tonnes) and mirtazapine (>1 tonne) in England rose significantly over the recent five years as presented in Tab. 7. Mirtazapine is distributed as a racemate and sertraline as one active enantiomer. Citalopram on the other hand is marketed as both a racemate and one enantiomer but prescription figures in England (Tab. 7) clearly indicate its main usage as racemate.

Pharmacodynamics and pharmacokinetics

Metabolism and elimination differ significantly within this group of chiral drugs. However, general pattern of significant metabolism leading to the elimination of usually >90% of parent compound and formation of several (sometimes active) metabolites can be observed.

Fluoxetine's two enantiomers are known to have similar potency in terms of the inhibition of 5-hydroxytryptamine (5-HT, serotonin) uptake. However, 5-HT uptake inhibition is known to differ in the case of the two enantiomers. *S*-fluoxetine has a higher duration of action than *R*-fluoxetine due to higher potency of its metabolite, norfluoxetine. Chiral norfluoxetine is formed as a result of demethylation of fluoxetine. *S*-norfluoxetine is 15 times more potent than *R*-norfluoxetine and 1.5 times more potent than *S*-fluoxetine[38, 41, 50, 65, 67]. Further metabolism can occur by *O*-dealkylation producing *p*-trifluoromethylphenol and hippuric acid. Less than 10% of the administered dose is excreted as unchanged drug[96].

In the case of citalopram, *S*-enantiomer is characterised by much higher potency in inhibition of 5-HT uptake. The metabolites of citalopram differ in their pharmacological activity and pharmacokinetic profile. Demethylation of citalopram leads to the formation of pharmacologically active desmethylcitalopram (*S*-enantiomer approx. 7 times less potent than the drug). Citalopram is distributed as a racemate and *S*-enantiomer. The single *S*-enantiomer has been marketed since 2002 as escitalopram[38, 41, 50, 67]. Metabolism of citalopram is presented in Fig. 12. Only about 12% of a daily dose is excreted unchanged in urine[96].

Paroxetine and sertraline on the other hand contain two chiral centres and are marketed as single stereoisomers. Sertraline isomers reveal selectivity of action. In the case of *trans* isomers, the (+)-enantiomer is a potent inhibitor of the uptake of serotonin, dopamine, and noradrenaline and the (-)-enantiomer is selective towards the inhibition of noradrenaline uptake. In the case of cis isomers, (+)-*1S,4S*-stereoisomer (marketed sertraline) shows potent and selective serotonin uptake inhibition activity[38, 126]. Both paroxetine and sertraline (and its main metabolite N-desmethylsertraline) are

readily metabolised and excreted mainly in the form of metabolites with <2% of the dose as parent compounds[96].

Trimipramine is administered as a racemate. Stereoselective metabolism is observed in the case of trimipramine with preferential *N*-demethylation in the case of (+)-trimipramine and 2-hydroxylation in the case of (-)-trimipramine[41, 65] (Fig. 13). Antidepressant activity of trimipramine results from (+)-enantiomer. (-)-Enantiomer is considered to be a depressant[126].

Mianserin (administered as a racemate) is a chiral tetracyclic antidepressant, in which case *S(+)*-enantiomer is pharmacologically active[127]. The main metabolites are: *N*-desmethylmianserin, 8-hydroxymianserin (both active) and mianserin *N*-oxide. Only about 5% of a dose is excreted in urine unchanged[96].

Mirtazapine is also marketed as a racemate. Both enantiomers reveal different pharmacological properties. For example, (+)-enantiomer shows at least 10 times higher affinity for postsynaptic α_2-adrenoceptors. In contrast (-)-enantiomer is 140 times more potent as an inhibitor of the 5-HT receptor[41]. Their pharmacokinetics are stereoselective (plasma levels of *R*(-)-enantiomer are 2-3 times higher than those of the *S*(+)-enantiomer)[65]. Metabolism of mirtazapine is also stereoselective. *R*(-)-mirtazapine is metabolised preferentially via *N*-glucuronidation, whereas *S*(+)-enantiomer is preferentially metabolised via 8-hydroxy oxidation, followed by conjugation with glucuronic acid[97].

Reboxetine on the other hand contains two chiral centres and is marketed as a racemate of only *RR*(-)- and *SS*(+)-enantiomers. *RR*(-)-reboxetine is more potent and reveals stereoselective difference in plasma protein binding than *SS*(+)-reboxetine[67]. Rolipram is introduced as racemate. *R*-enantiomer is more pharmacologically potent. Tranylcypromine is another chiral drug administered as racemic mixture of *1S,2R*(+) and *1R,2S*(-)-isomers. Both isomers reveal differences in their potency and action. Their pharmacokinetic behaviour is also different[67].

Venlafaxine is an inhibitor of reuptake of both serotonin and noradrenaline. It is marketed as a racemate. Both enantiomers have antidepressant properties[65]. Venlafaxine undergoes extensive first-pass metabolism in the liver, mainly to the active metabolite *O*-desmethylvenlafaxine. Other minor and less active metabolites include: *N*-desmethylvenlafaxine and *N,O*-didesmethylvenlafaxine. Venlafaxine is excreted in urine, in the form of free or conjugated metabolites (1-10% unchanged)[96].

Amitriptyline is a non-chiral tricyclic antidepressant. It undergoes *N*-demethylation with the formation of the secondary amine nortriptyline (also active). Hydroxylation of amitriptyline and nortriptyline is stereo- and enantioselective and leads to the formation of four isomeric alcohols. The (-)-*E*-10-hydroxy-amitriptyline and (-)-*E*-10-hydroxy-nortriptyline are the major products. Their disposition relating for example to glucuronidation is also enantioselective[128, 129].

Environmental occurrence and toxicity

SSRIs including fluoxetine, paroxetine and sertraline are the most commonly used antidepressants. As a result they are found in surface water at low ng per litre levels (Fig. 14). There is a risk of SSRIs reaching drinking water supplies. For example fluoxetine was quantified in finished water samples in the USA at low levels not exceeding 1 ng L^{-1} [130]. Due to extensive metabolism of SSRIs, their metabolites (e.g. norfluoxetine, norsertraline, desmethylcitalopram and nortriptyline) are also quantified in environmental matrices at comparable levels to parent compounds[131, 132] (Fig. 14). SSRIs are also known to resist most forms of degradation in the environment and tend to partition to sediments, where they might be persistent. Fluoxetine, sertraline, and their metabolites were found in fish suggesting possible bioaccumulation potential[2, 94].

SSRIs act by inhibiting the re-uptake of serotonin, a neurotransmitter involved in many mechanisms: hormonal, neuronal, food intake and sexual behaviour. Serotonin as a neurotransmitter exists in lower vertebrates and invertebrates, although, the effects associated with this transmitter

are different and possibly the effects of SSRIs can be also different. Serotonin mediates endocrine functions in aquatic organisms[2, 94, 133]. Acute and chronic toxicity of SSRIs in aquatic organisms, mainly fluoxetine, were studied by several research groups[133-140]. Fluoxetine is the most toxic human pharmaceutical reported so far. It's acute toxicity ranges from EC_{50} (48h, green alga) = 0.024mg L^{-1} to LC_{50} (48h, rainbow trout) = 2mg L^{-1} [2]. Data on chronic toxicity of SSRIs indicates their effect on reproduction of fish and invertebrates[2, 117, 138]. For example out of five SSRIs studied (fluoxetine, fluvoxamine, sertraline, paroxetine and citalopram) sertraline was found to be the most toxic after 7-8 days of exposure in *C. dubia* (LOEC=45µg L^{-1} and NOEC=9µg L^{-1}) but it was also confirmed that all five antidepressants could impact on both survival and reproduction[2,141]. Fluoxetine was also found to affect the growth and reproduction of freshwater molluscs[133, 139]. Nałęcz-Jawecki[142] studied toxicity of both fluoxetine and its main metabolite norfluoxetine. Both compounds were toxic to applied bioassays with LC_{50} around 0.5 mg L^{-1}. The compounds affected the protozoan *Spirostomum ambiguum* only slightly stronger than the crustacean *Thamnocephalus platyurus* in the 24 h lethality tests. Norfluoxetine was 50% more toxic than fluoxetine in both bioassays. The results give a strong indication of the importance of investigation of not only parent drugs but also their metabolites[142]. Other antidepressants also revealed high acute toxicity. For example, acute toxicity of citalopram was found to be 3.9 mg L^{-1} for daphnid (48h). In the case of amitriptyline: 0.78 mg L^{-1} for daphnid (48h) and 0.78 mg L^{-1} for fish (<96h). Studies with paroxetine resulted in the following acute toxicity: 0.58 mg L^{-1} for daphnid (48h) and 2 mg L^{-1} for fish (<96h). Acute toxicity of sertraline was found to be 0.12 mg L^{-1} for daphnid (48h)[10]. Acute toxicity of amitriptyline was found to be 1.15 mg L^{-1} for daphnid (24h)[143].

No relevant stereoselective toxicity studies were undertaken for the above mentioned antidepressants, with the exception of fluoxetine only. Toxic effects of fluoxetine enantiomers are species dependent: *S*-fluoxetine is more toxic than *R*-fluoxetine in *Pimephales promelas* and equal toxicity of both enantiomers is observed in the case of *Daphnia magna*[89]. Up to a 9.4-fold difference in toxicity between enantiomers was observed; *P. promelas* growth EC_{10}*s* (10% effect concentration) for *R*- and *S*-fluoxetine were 132.9 and 14.1 µg L^{-1}, respectively. This enantiomer dependant toxicity of fluoxetine is of vital environmental importance as fluoxetine is not released to the environment in a racemic form. According to limited studies undertaken by MacLeod et al.[84] untreated wastewater was found to be enriched with *R*(-)-fluoxetine, but due to biological wastewater treatment the enantiomeric ratio of fluoxetine's enantiomers changed and resulted in an enrichment of fluoxetine with *S*(-)-enantiomer, which is more potent and toxic to certain organisms. It is worth emphasising here that norfluoxetine, the main metabolite of fluoxetine, plays a vital role in its potency and possibly toxicity and therefore should always be considered in ecological risk assessment of this antidepressant.

Fluoxetine is only one of many chiral antidepressants which reveal (as discussed above) stereoselective potency in humans. There is, based on fluoxetine's example, a high possibility that other chiral racemic antidepressants will also show stereoselective transformation during wastewater treatment and/or in the environment, which might subsequently lead to enrichment of the drug with a more potent enantiomer and as a result in an increase of its overall toxicity. Primary results obtained for venlafaxine during wastewater treatment and published by Kasprzyk-Hordern et al.[88] support this hypothesis. Therefore, especially in the case of chiral antidepressants, distributed as racemates and characterised by significant differences in potencies of their enantiomers, extensive studies aiming to understand their enantiospecific fate and toxicity should be undertaken. As antidepressants are known to be extensively metabolised, the research should involve also their active, often chiral metabolites. Among antidepressants of the highest concern are: fluoxetine and its main active chiral metabolite norfluoxetine, citalopram and its active chiral metabolite desmethylcitalopram, trimipramine, mianserin, mirtazapine, rolipram and several others. Non-chiral antidepressants such as amitriptyline should also be considered as their metabolism might lead to the formation of chiral metabolites.

4.3.2.3. Sedative/hypnotics

Chiral sedative/hypnotics include: zopiclone, barbiturates (e.g. pentobarbital, hexobarbital, mephobarbital) and benzodiazepines (e.g. diazepam, oxazepam and temazepam), which are all marketed as racemates (Tab. 7).

Pharmacodynamics and pharmacokinetics

Zopiclone (Fig. 11) is a chiral hypnotic agent with stereoselectivity in pharmacokinetics and pharmacodynamics. The in vitro affinity of the *S*(+)-enantiomer for binding to the benzodiazepine receptor is 50 times higher than that of *R*(-)-zopiclone[126]. Its two main metabolites: *N*-demethylated derivative and *N*-oxide metabolite (active) are also chiral. The enantiomers' pharmacokinetics differs markedly and varies significantly between patients[119]. Only 5% of a dose of zopiclone is excreted unchanged in urine and about 16% appears in faeces, the remaining dose is excreted as metabolites[96].

Barbiturates exert their sedative and anaesthetic effects by potentiating the action of GABA and $GABA_A$ receptor. Pentobarbital, thiopental and secobarbital, thiamylal, mephobarbital, hexobarbital possess one chiral centre and are distributed as racemates. Methohexital on the other hand possesses two chiral centres[144]. Hexobarbital and mephobarbital reveal high stereoselectivity in their plasma concentrations after racemic doses[67]. Both thiopental and pentobarbital reveal greater volume of distribution and higher clearance in the case of *R*(+)-enantiomer. The *S*(-)-enantiomers of thiopental and pentobarbital are twice as potent as *R*(-)-enantiomers[144].

Benzodiazepines such as diazepam, as well as its metabolites: temazepam and oxazepam are chiral. Metabolism of benzodiazepines is stereoselective[126]. Diazepam is metabolised via *N*-demethylation, 3-hydroxylation and glucuronic acid conjugation. The major active metabolite of diazepam is desmethyldiazepam (nordiazepam); other metabolites include oxazepam and temazepam, both of which are active. Only small traces of unchanged diazepam are excreted in urine. Diazepam is a metabolite of ketazolam and medazepam. Temazepam is principally metabolised by glucuronic acid conjugation; demethylation to oxazepam occurs to a small extent. Less than 2% of the dose in excreted unchanged. Oxazepam is excreted mainly in urine as glucuronic acid conjugate with only traces of unchanged drug[96].

Environmental occurrence and toxicity

Only a few sedative/hypnotics were the subject of environmental investigation. Among them are benzodiazepines such as diazepam and barbiturates such as pento- and hexobarbital. They are present in aqueous samples at low ng L^{-1} levels. However, Peschka et al.[145] quantified barbiturates in the river Mulde in Germany at much higher levels reaching 5.4 μg L^{-1} in the case of pentobarbital and 5.3 μg L^{-1} and 0.1 μg L^{-1} in the case of butalbital and secobarbital respectively. Diazepam is believed to be marginally degraded in surface waters, and due to its relative hydrophobicity (logKow, 2.85) and pKa of 3.4, it will partition to river sediments, which suggests its high persistence. Oxazepam, on the other hand, due to its higher polarity, will be less likely to persist in river sediments[17]. Removal of benzodiazepines during wastewater treatment was reported to be limited[120, 146]. Acute toxicities of secobarbital and pentobarbital were found to be: 23.6 mg L^{-1} and 49.5 mg L^{-1} for fish (<96h) respectively[10]. Acute toxicity of diazepam denoted 4.3 mg L^{-1} for daphnid (24h)[143]. No relevant stereoselective environmental and ecotoxicity studies were undertaken for this group of compounds.

4.3.2.4. CNS stimulants and drugs used for ADHD

Methylphenidate (ritalin) is a chiral drug used in the treatment of attention deficit hyperactivity disorder. (+)-enantiomer is more potent than (-)-enantiomer[67]. (*R,R*)-Ritalin is used as an anti-ADHD, while (*S,S*)-ritalin is used as an antidepressant[147]. In the case of modafinil, an anti-narcoleptic drug, distributed as a racemate, stereoselective metabolism is observed and the clearance of the *S*(+)-enantiomer is three times higher than *R*(-)-enantiomer[62]. Annual prescription for both modafinil and methylphenidate in England accounts for >0.2 tonnes and is steadily

growing[95]. To the author's knowledge no environmental monitoring has been undertaken for these compounds to date.

4.3.2.5. Drugs used in neurological disorders

There are several chiral anticholinergic drugs used in Parkinson's disease. These are: procyclidine, trihexyphenidyl, biperiden, orphenadrine, ethopropazine, selegiline, levodopa, pergolide, apomorphine and entacapone (Fig. 11). Procyclidine, trihexyphenidyl and orphenadrine are marketed as racemates. Selegiline, apomorphine and levodopa are marketed as one active enantiomer (*R*(-)-selegiline, *R*-apomorphine, *L*-levodopa).

Among chiral antiepileptics are for example: mephenytoin, ethotoin, ethosuximide, vigabatrin, valnoctamide, levetiracetam, tiagabine and entacapone (Fig. 11). Antiepileptics are distributed in high quantities. In England annual prescription quantities for racemic ethosuximide and vigabatrin exceeds 0.5 and 1 tonne respectively. Levetiracetam marketed as *S*-enantiomer is annually prescribed in England in quantities exceeding 10 tonnes with a growing trend (Tab. 7).

Pharmacodynamics and pharmacokinetics

Among drugs used in Parkinsonism, *R*-procyclidine, *R*-trihexyphenidyl and (+)-biperiden are more potent in their ability to bind to muscarinic receptors. Minimal enantioselective pharmacokinetic data exists for this class of chiral drugs[67]. Selegiline (deprenyl) is distributed as *R*(-)-enantiomer and is metabolised to *R*(-)-methamphetamine and *R*(-)-amphetamine. Apomorphine is also distributed as one *R*-enantiomer as *S*-apomorphine is devoid of dopamine agonist activity[144]. Entacapone is a geometric isomer and is marketed in the *E*-isomeric form. It undergoes hepatic glucuronide metabolism as well as isomerisation in plasma and red blood cells to the *Z*-isomer[144].

The metabolism of antiepileptic mephenytoin in man is highly stereoselective. *S*-mephenytoin is rapidly metabolised by aromatic hydroxylation to 4-hydroxymephenytoin, which is rapidly eliminated in urine as a glucuronide conjugate. *R*-mephenytoin is metabolised through a different pathway, oxidative demethylation to form 5-phenyl-5-ethylhydantoin. Therefore the elimination kinetics of the two enantiomers is different. *S*-enantiomer has half-life of 4h and is eliminated in the form of 4-hydroxy metabolite within 24h. On the other hand 5-phenyl-5-ethhylhydantoin has a half-life of 5-6 days and accumulates as a result of repeated administration and reaches a steady state over 2-3 weeks[63]. Phenylethylhydantoin is active and chiral. Both parent drug and metabolite reveal high stereoselectivity in the plasma concentrations. However, its level decreases in poor metabolizes[67]. Phenytoin is another hydantoin derivative, which although not chiral itself is metabolised to chiral 5-(4-hydroxyphenyl)-5-phenylhydantoin, of which *R*-enantiomer is more potent. Similarly chiral ethotoin reveals stereoselectivity in pharmacokinetics[67]. Ethosuximide is distributed as a racemate without proven stereoselectivity in pharmacokinetic studies. Chiral vigabatrin (administered as racemate) on the other hand reveals high stereoselective pharmacokinetic effects and higher pharmacological activity in its *S*(+)-enantiomeric form[67]. Tiagabine is on the other hand distributed as *R*(-)-enantiomer due to much higher pharmacological activity of this enantiomer over *S*(+)-tiagabine. Levetiracetam is also administered as single *S*-enantiomer[144]. Oxcarbazepine, although achiral, is metabolised to active chiral 10-hydroxycarbazepine that shows stereoselectivity in the plasma concentrations but both its enantiomers are known to posses similar antiepileptic activities[67, 144]. Valproic acid (VPA) is also achiral, but its structural analogues: 4-yn-VPA and 4-en-VPA are chiral and have varying teratogenic potential: *R*(+)-4-yn-VPA<*R*(+)-4-en-VPA<VPA<*S*(-)-4-en-VPA<*S*(-)-4-yn-VPA. Valnoctamide on the other hand is chiral with two chiral centres and as a result four stereoisomers with varying pharmacokinetic stereoselectivity[67].

Rivastigmine, donepezil and galantamine are chiral drugs used for dementia. Rivastigmine was originally introduced as a racemate but later it was marketed as a 10 times more potent *S*(-)-enantiomer. In contrast, both enantiomers of donepezil reveal similar potency and therefore this drug is marketed as a racemate[144].

Environmental occurrence and toxicity

Very limited, if any, environmental research was undertaken for chiral drugs used in neurological disorders despite the high distribution of some drugs within this group. This is surprising taking into consideration the fact that achiral antiepileptics such as carbamazepine or gabapentin were widely studied is different environmental matrices. Achiral phenytoin was also studied and quantified in wastewater at levels reaching a few hundreds ng L^{-1} and was removed during wastewater treatment with only 44% efficiency[106]. Huerta-Fontela et al.[148] quantified phenytoin in wastewater at similar levels but, did not observe any removal of this compound during wastewater treatment. Phenytoin was also quantified in raw and finished drinking water at concentrations reaching 5.6 and 2 ng L^{-1} respectively[149]. Benotti et al.[130] also quantified phenytoin in several samples of raw, drinking finished water and the distribution system in the USA at levels reaching 29, 19 and 16 ng L^{-1} respectively. Unfortunately, its chiral metabolite, 5-(4-hydroxyphenyl)-5-phenylhydantoin, has never been a subject of environmental monitoring. Ecotoxicological studies concerning chiral antiepileptics are also limited in scope. Acute toxicity of orphenadrine was for example found to be high and accounted for 10.6 mg L^{-1} for daphnid (48h) and 4.3 mg L^{-1} for fish (<96h)[10]. Similarly to other groups of chiral drugs no stereoselective studies were undertaken in terms of their occurrence in the environment and environmental toxicity.

4.3.3. Cardiovascular drugs

4.3.3.1. Beta-adrenoceptor blocking drugs

Beta-blockers are well understood in terms of their stereoselective pharmacokinetics and pharmacodynamics in humans. They posses at least one chiral centre and are characterised by a high degree of enantioselectivity to the *β*-adrenergic receptor. With the exception of timolol (marketed as *S*-enantiomer), they are clinically administered as racemates (Tab. 8). Propranolol, metoprolol, esmolol, pindolol and acebutolol with one chiral centre, are marketed as racemate of two isomers (Fig. 15). Labetalol with two chiral centres is marketed as a racemate of four isomers. Nadolol has three chiral centres[150]. Beta-blockers are widely distributed in the world. Annual prescription of several beta-blockers in England accounts for >2 tonnes with atenolol being prescribed in the highest quantities exceeding 30 tonnes/year (Tab. 8). In Germany metoprolol was consumed in 93 tonnes in 2001 and an increasing trend of consumption has been observed over recent years (68 t/1999; 79 t/2000 and 93 t/2001)[2].

Pharmacodynamics and pharmacokinetics

Pharmacological action of beta-blockers (binding with beta-adrenoceptors) in humans is highly stereoselective. *S*(-)-enantiomers reveal much higher cardiac beta-blocking potency than *R*(+)-enantiomers in most beta-blockers, with an activity ratio being in the region of *S*:*R* = 33 to 530. On the other hand *R*(+)-enantiomers have higher activity in blocking β_2 receptors in ciliary processes. In the case of sotalol *R*(-)-enantiomer has much higher beta-blocking activity. Labetalol with two chiral centres reveals both beta- and alpha- receptor blocking activity. *RR*-isomer is responsible for beta-blocking activity and *SR*-isomer is responsible for alpha-blocking activity. On the other hand *RS*- and *SS*-isomers display weak antagonistic activities against alpha and beta-receptors[150, 151].

Stereoselectivity in pharmacokinetics is characteristic for beta-blockers. The elimination of most beta-blockers takes place through hepatic metabolism (characteristic for more hydrophobic compounds such as propranolol and metoprolol) and/or renal excretion (characteristic for more hydrophilic drugs such as atenolol and nadolol, which are excreted, unchanged). Metabolism of beta-blockers reveals high stereoselectivity. For example propranolol as shown in Fig. 16, is metabolised through three main pathways of glucuronidation, ring hydroxylation and side chain oxidation and is selective for less active *R*(+)-enantiomer resulting therefore in higher plasma concentrations of *S*(-) enantiomer in human. The ring hydroxylation pathway shows selectivity for *R*(+)-propranolol. Formed hydroxypropranolol is further conjugated with glucuronic acid, favouring *S*(-)-enantiomer, or with sulphate favouring *R*(+)-enantiomer. *N*-dealkylation favours *R*(+)-

enantiomer at low concentrations of propranolol, or *S*(-)-enantiomer at high concentrations of propranolol. Glucuronidation pathway favours *S*(-)-propranolol[97, 150].

Environmental occurrence and toxicity

Among beta-blockers, propranolol, metoprolol and atenolol are the most widely reported in environmental studies concerning pharmacologically active compounds. Due to their high usage, they are frequently quantified in surface water at concentrations reaching a few hundreds ng L^{-1} (Fig. 17). Atenolol was also quantified in several finished drinking water samples in the USA at levels reaching 18 ng L^{-1} [130]. Beta-blockers are removed during wastewater treatment with varying low to medium efficiency. For example atenolol removal rate in Italian WWTPs varied form 0 to 76% and was season dependent[108]. Kasprzyk-Hordern et al.[9] reported much better efficiency of beta-blockers removal during activated sludge treatment (33-81%) when compared with trickling filters (0-69%). Atenolol was characterised by the highest removal efficiency as opposed to metoprolol and propranolol[9]. Low to moderate beta-blockers removal efficiency during activated sludge treatment has been observed by others: 11, 64, 76 and 66% in the case of metoprolol, acebutolol, atenolol and sotalol respectively[152]. A similar pattern was observed by Wick et al.[120]. Limited enantioselective analysis of beta-blockers in environmental samples was also undertaken and is reviewed in paragraph 4.2.1. Several authors reported stereoselective biological degradation of beta-blockers during WWTP treatment and in the aqueous environment[79, 82, 83]. Propranolol for example has been found to be racemic in wastewater influent. Effluent in contrast was enriched with *S*(-)-propranolol, which is known to have higher toxicity towards *Pimephales promelas* than its antipode[84, 93].

Beta-blockers act by competitive inhibition of beta-adrenergic receptors and are used in the treatment of high blood pressure and to treat patients after a heart attack. The adrenergic system plays a vital part in many physiological functions such as regulation of the heart oxygen need and beating, vasodilation mechanisms of blood vessels and bronchodilation. It also interacts with carbohydrate and lipid metabolisms, mainly as a response to stress needs such as starvation. Side effects of beta-blockers involve bronchoconstriction and disturbed peripheral circulations[2]. Some beta-blockers such as propranolol cross the blood-brain barrier[94]. Beta-adrenoceptors are found in vertebrates and many invertebrates. Acute toxicity of beta-blockers has not been widely studied, although it is known that propranolol is the most toxic. Phytoplankton and zooplankton are more sensitive than fish (*Ceriodaphnia dubia*, EC_{50}(48h)=0.8mg L^{-1}; *D. magna*, EC_{50}(48h)=1.6mg L^{-1}; *Synechococcus leopolensis* EC_{50}(96h)=0.67mg L^{-1}). As fish contains beta-receptor in heart, liver and probably in reproductive tissues, propranolol was found to show chronic toxicity in both the cardiovascular and reproductive systems. The lowest-observed-effect-concentration (LOEC) of propranolol affecting reproduction in *C. dubia* was 250 μg L^{-1}. Reproduction was also affected in *H. azteca* at 100 μg L^{-1} [2, 117]. Chronic exposure of *D. magna* to propranolol (9 days) resulted in a significant reduction in heart rate, fecundity and biomass with LOECs values of 55, 110 and 440 μg/L respectively while chronic exposure to metoprolol showed LOECs of 12.5 mg/L (body mass) and 6.15 mg/L (reproduction)[212]. A multigenerational study of Daphnia magna in the presence of metoprolol at environmentally relevant concentrations revealed a decreased body length and reduced number of offspring[213]. Limited information exists regarding the enantioselective toxicity of propranolol as discussed in paragraph 4.2.2.

4.3.3.2. Anticoagulants

Among chiral oral anticoagulants marketed as racemates are: warfarin, phenprocoumon and acenocoumarol (Fig. 15, Tab. 8).

Pharmacodynamics and pharmacokinetics

Warfarin is administered as racemate despite the fact that *S*(-)-warfarin is more potent[35]. Warfarin enantiomers are extensively metabolised by liver. Stereoselective pharmacokinetics is observed in the case of this compound. The metabolism of warfarin is qualitatively different. *R*-warfarin is oxidised to 7-hydroxywarfarin and reduced to *R,S*-warfarin alcohol. *S*-warfarin (a more active

enantiomer with 3-5 times higher anticoagulant potency[50]) on the other hand is oxidised to 7-hydroxywarfarin and reduced to *S,S*-warfarin alcohol. *S*-warfarin can also be metabolised to 6-hydroxywarfarin. A number of drugs may interact with the metabolism of warfarin enantiomers, e.g. sulphaphenazole and tolbutamide are competitive inhibitors of *S*-warfarin hydroxylation[67].

In the case of phenprocoumon, *S*-enantiomer is 1.5-2.5 times more potent than *R*-enantiomer[50]. Interaction with serum albumin, tissue distribution, as well as pharmacokinetic and pharmacodynamic properties of phenprocoumon are also stereoselective[153]. Phenprocoumon is excreted almost entirely as a glucuronide conjugate with less than 10% of the dose as unchanged drug[96].

Environmental occurrence and toxicity

Warfarin has received minimal attention by environmental research groups. In limited studies undertaken it was not quantified in surface water[154]. Acute toxicity of warfarin and warfarin sodium salt was found to be 342 and 17 mg L^{-1} for daphnid (48h) respectively and 12 mg L^{-1} for fish (<96h)[10]. No stereoselective studies were undertaken for anticoagulants in the environment.

4.3.3.3. Calcium channel blockers

The majority of calcium channel blockers are chiral and most of them are distributed as racemates (Tab. 8). There are three main groups of calcium channel blockers: dihydropyridines (e.g. amlodipine, nircadipine, nimodipine, nisoldipine and felodipine), phenylalkylamines (e.g. verapamil) and benzothiazepines (e.g. diltiazem) (Fig. 15)[155]. The annual prescription quantities of selected chiral calcium channel blockers in England is presented in Tab. 8.

Pharmacodynamics and pharmacokinetics

Many dihydropyridines have one or more chiral centers and are administered as a racemate. Enantiomers of dihydropyridines are characterised by different pharmacological and pharmacokinetic properties. For example (+)-nicardipine is three times as potent in increasing vertebral blood flow and lowering mean blood pressure as the (-)-isomer[155, 156]. The *S*(+)-enantiomer of nilvadipine is also about 100 times more potent in relaxing potassium-induced contractions of isolated dog coronary arteries than the *R*(-)-enantiomer[156]. A similar effect is observed in the case of amlodipine, where *S*-enantiomer is potent[151]. Species or sex-dependent stereoselective disposition of several dihydropyridines such as: nilvadipine in rats, dogs, and humans, felodipine in rats, dogs, and humans, and lemildipine in rats has been also observed, while negligible species and sex differences have been found for nisoldipine in dogs, rats, and mice. For example *S*-enantiomer of nilvadipine was more rapidly eliminated in humans, while the opposite was true in dogs and rats[155, 156].

Verapamil is marketed as a racemate and is used in both human and veterinary treatment. Most of the cardiovascular effects of racemic verapamil are mediated by *S*(-)-enantiomer. *S*(-)-verapamil has more potent vasodilation and cardiac depressant properties. On the other hand *R*(+)-enantiomer is predominantly a vasodilating drug[147]. Clearance and plasma protein binding is stereoselective in both humans and animals. Moreover, pharmacokinetics of verapamil is species dependant and can be affected by the presence of other drugs[48]. Metabolism of verapamil (Fig. 18) is stereoselective and favours the more active *S*(-)-enantiomer[97, 151, 157].

Diltiazem (distributed as *cis*(+)-stereoisomer), after oral administration undergoes extensive first-pass hepatic metabolism via deacetylation, N-demethylation, O-demethylation and oxidative deamination. Only 2-4% of administered dose appears unchanged in urine[96].

Environmental occurrence and toxicity

No or limited reports exist on the presence of calcium channel blockers in environmental matrices. Hummel et al.[146] reported relatively high concentrations of verapamil in wastewater influent (3.1 µg L^{-1}), effluent (0.51 µg L^{-1}) and in surface water (6 ng L^{-1}). Acute toxicity of verapamil was found to be 7 mg L^{-1} for daphnid (48h) and 6.2 mg L^{-1} for fish (<96h)[10]. Diltiazem was found in surface

waters at ng L^{-1} levels reaching 200 ng L^{-1} (Fig. 17). The acute toxicity of diltiazem was reported to be 8.2 mg L^{-1} for daphnid (96h)[158], which indicates that this compound is toxic to daphnid. Similarly to other groups of chiral drugs, and despite the fact that most calcium channel blockers are distributed as racemates, no stereoselective occurrence and toxicity studies were undertaken for this group of compounds.

4.3.3.4. Anti-arrhythmic drugs

Many antiarrhythmic drugs are chiral and are distributed as racemates. Among them are: disopyramide, encainide, flecainide, mexiletine, propafenone and tocainide (Fig. 15; Tab. 8). These drugs are distributed in communities in high quantities accounting in England for >1.5 tonnes/year in the case of flecainide.

Pharmacodynamics and pharmacokinetics

Antiarrhythmic drugs exert their effects mainly through the blockade of sodium channels. Except for flecainide and encainide, significant stereoselectivity in one or more of the pharmacological actions of chiral antiarrhythmic drugs (with the activity of one enantiomer 100-fold or higher) can be observed. Absorption of antiarrhythmic drugs seems not to be stereoselective but distribution, metabolism and renal excretion reveal significant stereoselectivity[157]. For example *S*(+)-enantiomer of disopyramide is much more potent as an antiarrhythmic drug. It is characterised by lower plasma and renal clearance than *R*(-)-enantiomer[147]. In the case of tocainide and mexiletine *R*(-)-enantiomers are four and two times respectively more potent than their antipodes in sodium channel blocking activity. In the case of propafenone, despite showing no stereoselectivity in sodium channel blocking activity, its *S*(+)-enantiomer is almost 100 times more potent than *R*(-)-enantiomer in the blockage of human β-receptors[157].

Hepatic metabolism plays a major role in the elimination of antiarrhythmic drugs. In the case of disopyramide, metabolism is responsible for the elimination of about 45% of administered dose with the only identified metabolic pathway being stereoselective mono-*N*-dealkylation favouring *S*(+)-enantiomer[157]. The major metabolite, *N*-monodesisopropyldisopyramide, is about one half as active as disopyramide. About 50-60% of a dose is excreted in the urine as unchanged drug[96]. The elimination of flecainide may account for 60% of administered dose. The major metabolism pathways in humans involve the formation of *m*-*O*-dealkyl-flecainide and *m*-*O*-dealkyl-flecainide lactam (Fig. 19). Both pathways might be impaired in poor metabolisers. This inhibition is stereoselective and favours *R*(-)-enantiomer[157]. Metabolism of mexiletine accounting for 90% of administered dose is also stereoselective and favours *R*(-)-enantiomer. The major pathways are aliphatic and aromatic hydroxylation leading to hydroxymethyl-mexiletine and *m*- or *p*-hydroxy-mexiletine. A degree of stereoselectivity with the R/S ratio of 11 is observed in the formation of *N*-hydroxy-mexiletine glucuronide[157]. In humans, propafenone undergoes extensive metabolism (accounting for 100% of administered dose) with the formation of 5-hydroxy- and *N*-dealkyl-propafenone as the main metabolites. These two metabolism pathways are not stereoselective. Stereoselectivity of propafenone metabolism results therefore from other minor pathways[157]. 60% of administered dose of tocainide is eliminated through hepatic stereoselective metabolism leading to the formation of a glucuronide conjugate of *N*-carboxy-tocainide favouring *R*(-)-enantiomer[157].

Environmental occurrence and toxicity

Limited environmental studies were undertaken for this group of drugs. Furthermore, no research concerning their stereoselective toxicity, environmental occurrence and fate has been reported. Research efforts should therefore concentrate on understanding the fate of these pharmaceuticals in the environment and assessment of their ecotoxicity should be also considered. Due to the common formation of active metabolites, their environmental impact should also be estimated.

4.3.3.5. Other cardiovascular drugs

Among chiral angiotensin-converting enzyme (ACE) inhibitors are ramipril, enalapril, lisinopril, quinalapril and several others (Fig. 15). ACEs have been developed and are marketed as single optical isomers as only *S*-enantiomer is pharmacologically active[54]. ACEs have been hardly studied in the environment. Enalapril is the most commonly reported (Fig. 17). Its removal during WWTP treatment accounted for 4-100% and was found to be season dependant[108].

Chiral angiotensin II receptor antagonists include: losartan and valsartan[94] (Fig. 15). Valsartan is marketed as a single active *S*-enantiomer. Losartan's activity resides only in *R*-enantiomer[159]. Valsartan was quantified in environmental matrices such as surface water at ppt levels (Fig. 17). The acute toxicity of losartan was found to be 331 mg L^{-1} for daphnid (48h), 245 mg L^{-1} for algae (24h) and 929 mg L^{-1} for fish (<96h)[10].

There are two types of lipid-regulating drugs: statins and fibrates. They are used to decrease the concentration of cholesterol (statins and fibrates) and triglycerides (fibrates) in the blood plasma[2, 94]. Statins do not only affect mammals but also interfere with juvenile hormone synthesis in insects and also have an adverse effect on algae and plants. Atorvastatin and lovastatin were found to have herbicidal activity in duckweed (*Lemna gibba*) with EC_{50} of 26 and 33 μg L^{-1} respectively[117]. Both acute and chronic toxicity data on this group of compounds is rare[2, 94]. Statins such as: atorvastatin, simvastatin, pravastatin, lovastatin and rosuvastatin are marketed as single enantiomers (Tab. 8). Their annual usage accounts for tens of tonnes in England. Due to high usage they are quantified in environmental samples at ng L^{-1} levels (Fig. 17).

4.3.4. Respiratory drugs

4.3.4.1. Bronchodilators

Bronchodilators open up the airways of the lungs by relaxing the muscles in the air tubes[94]. Among chiral *β*-agonists used in the treatment of asthma are: salbutamol (albuterol), bambuterol, isoprenaline, orciprenaline, clenbuterol, formoterol, fenoterol and terbutaline (Fig. 20, Tab. 9). Other anti-asthmatic drugs include: zileuton, ipratropium and montelukast (marketed as a single *R*-enantiomer). All *β*-agonists are marketed as racemates despite the fact that *R*-enantiomers are known to be much more potent than *S*-enantiomers. Trimethoquinol is an exception where *S*-enantiomer is more potent[67, 160]. Their metabolism is stereoselective.

Pharmacodynamics and pharmacokinetics

Chiral *β*-agonists are mainly subject to phase 2 metabolism (sulphation and glucuronidation). Sulphation is catalysed by a monoamine form of phenol sulphotransferase. *S*-enantiomers of chiral *β*-agonists reveal much higher affinity towards this enzyme than their eutomers. However, there are two exceptions: albuterol and salmeterol, in the case of which *R*-enantiomer shows higher affinity. Enantioselectivity in renal clearance of chiral *β*-agonists was also reported in the case of albuterol and terbutaline. While in the case of albuterol renal clearance is two-fold higher for *R*-enantiomer, the opposite situation is observed in the case of terbutaline[161].

Salbutamol is administered as both a racemate and single *R*-enantiomer. Its bronchodilator activity resides in *R*(-)-enantiomer. *S*(+)-enantiomer, on the other hand indirectly antagonises the benefits of *R*(-)-salbutamol. Pharmacokinetics is known to be stereoselective in the case of salbutamol. *S*(+)-salbutamol is cleared more slowly than its *R*(-)-enantiomer and therefore the potentially harmful enantiomer will be more likely to accumulate. Due to pharmacokinetic and pharmacodynamic differences between salbutamol enantiomers a successful racemic switch was undertaken from racemic albuterol to *R*(-)-albuterol (levabuterol)[122, 160, 162, 163]. About 60-90% of an orally administered dose is excreted in urine, of which 50% is unchanged salbutamol and 50% is the 4'-*O*-sulfate of salbutamol[96].

Formoterol has two chiral centres and is introduced as the racemic mixture of active *RR*- and inactive *SS*-enantiomer. *RR*-enantiomer is 1000 times more potent at the human β_2-adrenoceptor than the *SS*-isomer. *SS*-isomer, similarly to salbutamol, may be antagonistic to *RR*-formoterol. In

the case of terbutaline, *R*(+)-isomer has higher activity in *β*-adrenergic receptor antagonist action[164]. Formoterol is eliminated mainly through glucuronidation, which is stereoselective and favours *SS*-isomer[161]. Bambuterol is a prodrug of terbutaline. The drug itself is inactive, but it is metabolized enzymatically in vivo by Butyryl Cholinesterase (BuChE) into the active compound terbutaline (Fig. 20). *R*-bambuterol is at least two times more potent than *S*-bambuterol in the treatment of asthma. On the other hand, *S*-bambuterol was inactive in the treatment of asthma but has more adverse cardiac toxic effects than *R*-bambuterol[165]. Other chiral anti-asthmatic drugs also reveal stereoselective metabolism. For example, montelukast undergoes stereoselective oxidative biotransformation leading to several isomers. Zileuton also undergoes stereoselective glucuronidation at an *N*-hydroxy group. Zileuton also reveals stereoselective pharmacokinetics, with concentrations of the *R*-enantiomer exceeding those of the antipode[161].

Environmental occurrence and toxicity

Salbutamol is the most widely studied bronchodilator in the environment and quantified in environmental aqueous samples across Europe at ng L^{-1} levels reaching 500 ng L^{-1} [8, 9, 166-169]. Low removal efficiencies were observed in Italian WWTPs and accounted for 0-12%[108]. Kasprzyk-Hordern et al.[9] observed much higher removal efficiencies of salbutamol accounting for 66% in the case of trickling filters and 89% in the case of activated sludge treatment. Jones et al.[170] also observed >90% removal of salbutamol during activated sludge treatment. The acute toxicity of salbutamol was found to be 51 mg L^{-1} for daphnid (48h)[10]. Limited enantioselective analysis of salbutamol in environmental samples has been undertaken and is reviewed in paragraph 4.2.1. Unfortunately, to the author's knowledge no stereoselective analysis of the ecotoxicity of salbutamol has been reported to date.

4.3.4.2. Antihistamines

Antihistamines block histamine H1 at the receptor site[94]. Cetirizine (Fig. 20, Tab. 9) is used for the treatment of allergic rhinitis (hay fever) and is distributed as a racemate. Levocetirizine (*R*(-)-enantiomer) on the other hand is less sedating than the racemate cetirizine[147]. Levocabastine has been found to be 4-90 times more potent than dextrocabastine in guinea pigs. Chlorpheniramine is available as a racemate. *S*(+)-enantiomer of chlorpheniramine was found to have higher maximum drug levels and lower clearance and volume of distribution. Pyranenamine has two chiral centres. *SS*-isomer was found to have a much more potent inhibitor effect on the allergic response when compared with the *RR*-isomer[164]. Fexofenadine contains one asymmetric carbon and is distributed as a racemate. Its enantiomers have equal potencies but different pharmacokinetics e.g. plasma concentrations of *R*(+)-fexofenadine are higher than for *S*(-)-enantiomer. Clearance of *S*(-)-fexofenadine is also significantly higher that *R*(+)-enantiomer[171]. Unfortunately, to the author's knowledge no detailed environmental data on the occurrence and toxicity of antihistamines is available. Cetirizine has been recently studied by Huerta-Fontela et al.[148] in WWTPs. It has been found at high concentrations in raw wastewater exceeding μg L^{-1} levels. In WWTPs effluents it has been quantified at levels reaching 500 ng L^{-1}.

4.3.5. Gastro-intestinal system - proton pump inhibitors

Proton-pump inhibitors inhibit gastric secretion by blocking the H^+K^+-ATPase in the proton pump. Because the proton pump is the final pathway for the secretion of hydrochloric acid by the parietal cells in the stomach, its inhibition dramatically decreases the secretion of hydrochloric acid into the stomach and alters gastric pH[94]. Chiral proton pump inhibitors such as omeprazole, pantoprazole, rabeprazole and lansoprazole (Fig. 20), which are used in the treatment of gastrointestinal disorders, possess a chiral sulphur atom and not carbon. Their metabolism and elimination are stereoselective[67, 173]. These drugs are administered as racemates, with the exception of esomeprazole, *S*(-)-enantiomer of omeprazole (Tab. 9). The development of esomeprazole was based on the unique metabolic properties of *S*-enantiomer from racemate. Omeprazole and esomeprazole act by blocking the final stage in the acid secretion process. This is done indirectly by their metabolite, achiral sulphonamide, which is the actual active inhibitor. Two enantiomers are

subject to the same metabolic transformations (Fig. 4) but there are quantitative differences, which result in the superiority of *S*-enantiomer over *R*-enantiomer or racemate. Hydroxylation is responsible for 98% of the total intrinsic clearance of the *R*-enantiomer and only 70% of *S*-enantiomer. Sulphone formation is responsible for 2% of the total intrinsic clearance of the *R*-enantiomer and 30% of *S*-enantiomer. The total intrinsic clearance of *S*-omeprazole is one-third of that of the *R*-enantiomer in humans. The more advantageous pharmacokinetics for *S*-omeprazole over *R*-enantiomer or racemate result therefore from the lower metabolic clearance and lower variability, which lead to more effective gastric acid inhibition[174].

Both the *R*- and *S*-enantiomers of lansoprazole are equally pharmacologically potent. However, significant differences in pharmacokinetics are observed for the two enantiomers due to stereoselective metabolism. Lansoprazole is extensively metabolized in the liver with the formation of two major metabolites: inactive 5-hydroxylansoprazole (chiral, pathway favouring *S*-enantiomer) and lansoprazole sulphone (achiral). Because *R*-lansoprazole is less influenced than *S*-enantiomer by metabolism pathway leading to the formation of inactive 5-hydroxylansoprazole, it is considered to be the main active compound[173, 175]. Similarly, pantoprazole reveals enantioselective pharmacokinetics resulting from enantioselective metabolism. In rats, *S*-pantoprazole is favoured for the formation of pantoprazole sulphone and 6-hydroxy-pantoprazole, whereas *R*-pantoprazole is favoured for the formation of 4'-*O*-demethyl-pantoprazole sulphide[92] (Fig. 21).

Among proton pump inhibitors omeprazole has received the greatest (although still limited) attention in environmental studies[15, 166, 167, 176]. The acute toxicity of omeprazole was found to be 88 mg L^{-1} for daphnid (48h). The acute toxicity of lansoprazole was found to be 22 mg L^{-1} for daphnid (48h) and 18 mg L^{-1} for fish (<96h)[10]. The above mentioned acute toxicity levels indicate that proton pump inhibitors can be harmful to aquatic organisms. Additionally, they are expected to be present in the environment due to the high usage of these pharmaceuticals worldwide. As presented in Tab. 9, these pharmaceuticals are prescribed in tens of tonnes annually in England only. Furthermore, despite the introduction of esomeprazole, racemic omeprazole is still being prescribed at much higher (ca 4 times) quantities than its chiral analogue (Tab. 9). Research efforts should therefore concentrate on this group of chiral drugs, especially because no stereoselective studies on environmental fate and ecotoxicity have been undertaken to date.

4.3.6. Antimicrobials

Within the group of antimicrobials there are many chiral drugs: antibiotics (e.g. ofloxacin, sulfamethoxazole), antifungals (e.g. ketoconazole) (Fig. 20) and antiviral drugs (e.g. valacyclovir).

There are several fluooroquinolone antibiotics that are chiral and introduced as racemate. These are: ofloxacin, tosufloxacin and clinafloxacin. In the case of racemic ofloxacin, only *S*(-)-enantiomer displays a high antibacterial effect against gram-positive and gram-negative organisms (*S*(-)-enantiomer, marketed as levofloxacin, is over 100 times more potent than *R*(+)-enantiomer). Furthermore, (+)-enantiomer of tosufloxacin reveals 10 to 100 times higher in vitro level of antibacterial activity than its (-)-enantiomer. Low to moderate pharmacokinetic stereoselectivity was observed in the case of chiral fluoroquinolones[67]. Semi-synthetic antibiotics manufactured by fermentation such as penicillins and cephalosporins are overwhelmingly marketed as single isomers[54].

Among chiral antimalarials administered as racemate are: primaquine, mefloquine, halofantrine, quinacrine, lumefantrine, chloroquine and hydroxychloroquine. Chiral antimalarial drugs reveal stereoselectivity in pharmacokinetics and pharmacodynamics and therefore the adverse effects of these drugs can be stereoselective. Chiral metabolites are also formed from some chiral antimalarial drugs although stereoselective aspects of the pharmacokinetics of the metabolites are not well understood[67, 177]. Hydroxychloroquine for example is a racemic drug which is metabolised with the formation of three main chiral metabolites: desethylchloroquine, desethylhydroxychloroquine and bisdesethylchloroquine. Distribution, elimination and metabolism of hydroxychloroquine are enantioselective e.g. renal clearance of *S*-hydroxychloroquine is higher that *R*-enantiomer[178].

There are several classes of antifungal drugs. Chiral imidazoles (ketoconazole, econazole, bifonazole, fenticonazole, miconazole, isoconazole, tioconazole and sulconazole) and triazoles itraconazole and terconazole) constitute an important class. Most imidiazole and triazole antifungals are marketed as racemates. Ketoconazole has two chiral centres and is marketed as a racemate of *cis*-configuration *((+)-2R, 4S* and *(-)-2S, 4R*). Pharmacokinetics of ketoconazole is stereoselective e.g. enantioselectivity in plasma protein binding is significant[179]. Terconazole, similarly to ketoconazole, has two chiral centres and is also administered as a racemic mixture of *cis*-configuration. Itraconazole (ITC) has three chiral centres and is marketed as a racemic mixture of four stereoisomers in *cis*-configuration. Its major metabolite, hydroxyitraconazole has four chiral centres, is also highly active and can reach levels 2–3 times higher than that of ITC[180, 181].

4.3.7. Antineoplastics

Cyclophosphamide and ifosfamide (Fig. 20) are commonly used chiral drugs in cancer treatment. Their chiral centre is not a carbon atom but phosphorous. In the case of both drugs, not parent molecules but their metabolite reveals pharmacological action. This is phosphoramide mustard. There is little or no stereoselectivity of cyclophosphamide enantiomers in human plasma, metabolism and excretion of cyclophosphamide. On the other hand metabolism of ifosfamide enantiomers can have toxicological significance[58, 67]. Ifosfamide is marketed as a racemate and its metabolism is enantioselective[178]. Other chiral antineoplastics include: aminoglutethimide (used as a racemate in the treatment of breast cancer) and bicalutamide (used in the treatment of prostate cancer). (+)-aminoglutethimide reveals higher antitumor activity. Its metabolism and clearance are stereoselective. In the case of bicalutamide, *R*-enantiomer has higher pharmacological activity. Similarly to aminoglutethimide, metabolism and elimination are stereoselective with much faster clearance of *S*-enantiomer[67].

The acute toxicity of cyclophosphamide was found to be as follows: 70 mg L^{-1} for fish, 1795 mg L^{-1} for daphnid and only 11 mg L^{-1} for algae[3]. Both cyclophosphamide and ifosfamide are non-biodegradable during wastewater treatment and also when present in the aqueous environment. They were detected by Buerge et al.[182] in untreated and treated wastewater at concentrations of <0.3-11 ng L^{-1}. In surface waters, concentrations ranged from 50 to 170 pg L^{-1} and were thus several orders of magnitude lower than the levels at which acute ecotoxicological effects have been reported. However, due to a lack of studies on chronic effects on aquatic organisms and data on occurrence and effects of metabolites, a final risk assessment cannot be made[182] (Buerge et al., 2006).

4.3.8. Illicit drugs

Most illicit drugs are chiral compounds (Fig. 20). Among them are plant-derived substances (e.g. cannabis, cocaine and heroin) and synthetic drugs (e.g. amphetamine, methamphetamine and related designer drugs). Their enantiomers reveal different potency and are often characterised by stereoselective disposition in the body. *R,R*(+)-LSD is for example over 20 times more psychoactive than (-)-LSD[95]. Cocaine, similarly to heroin, naturally occurs in the form of *1R,2R,3S,5S*(-)-cocaine. (+)-Cocaine (the unnatural enantiomer) is inactive. Both metabolism and toxicity of (+)- and (-)-cocaine were found to be stereoselective[183]. In cannabinoids, the natural delta-1-THC and delta-6-THC have a (3*R*,4*R*) configuration and a negative rotation. Synthetic (+)-isomers are much less active, e.g. (+)-delta-1-THC is ca 13 to 230 times less active than the (-)-isomer in cannabimimetic activity[211].

Amphetamines belong to the group of central nervous system stimulants. Among them are: amphetamine, methamphetamine, 3,4-methylenedioxymethamphetamine (MDMA), 3,4-methylenedioxyamphetamine (MDA) and 3,4-methylenedioxy-*N*-ethylamphetamine (MDEA). Amphetamine and methamphetamine have some limited therapeutic use in narcolepsy and attention deficit hyperactivity disorder, but most are manufactured in clandestine laboratories[30]. Amphetamine is also formed as a metabolite of methamphetamine and several prescription drugs such as selegiline. Amphetamines are characterised by one asymmetric carbon centre and exist in

the form of two enantiomers, which significantly differ in potency, e.g. *S*(+)-amphetamine has twice as high stimulant activity than *R*(-)-amphetamine. However, *R*(-)-amphetamine has been reported to be as effective as the *S*(+)-enantiomer in the development of the psychotic syndrome. MDMA is used as a racemate, although, similarly to amphetamine its *S*(+)-enantiomer is much more potent as a CNS agent than is *R*(-)-MDMA[95].

Environmental occurrence and toxicity

There are several illicit drugs that have been identified in the aquatic environment. Cocaine and its metabolites belong to the group of the most studied illicit drugs in the environment. It is usually quantified in surface water at concentrations <50 ng L^{-1} (Fig. 22). Benzoylecgonine, its major metabolite, is found in surface water at much higher levels reaching a few hundreds ng L^{-1} (Fig. 22). Other metabolites of cocaine quantified in surface waters include: norbenzoylecgonine, norcocaine and cocaethylene. Measurable levels of cocaine and its metabolites in surface waters are linked with insufficient communal wastewater treatment, as both cocaine and its metabolites are present in raw and treated wastewater at high concentrations reaching in the case of benzoylecgonine 10 µg L^{-1} and 3 µg L^{-1} in wastewater influent and effluent respectively (Fig. 22). Amphetamines, another group of central nervous system stimulants, constitute the second group of the most studied illicit drugs. Amphetamines are frequently found in rivers across Europe at levels reaching 50 ng L^{-1} (Fig. 22). Amphetamine is the most abundant drug within the group of amphetamines and is found in surface water and wastewater at the highest levels. Concentrations of amphetamines in wastewater were found to vary between a few ng L^{-1} and <5 µg L^{-1} in different wastewater treatment plants and different countries and are a reflection of local drug abuse trends (Fig. 22). The most abused delta-9-tetrahydrocannabinol (THC), an active constituent of cannabis, its major metabolite, 11-nor-9-carboxy-delta-9-tetrahydrocannabinol (THC-COOH) and 11-hydroxy-THC (OH-THC) have also been quantified in rivers and/or wastewater at low ng L^{-1} levels (Fig. 22). Other studied illicit drugs include: LSD and its metabolites: 2-oxo-3-hydroxy lysergic acid diethylamide (O-H-LSD) and nor-LSD. This potent hallucinogen has been quantified in wastewater at single ng L^{-1} reaching 3 ng L^{-1} (Fig. 22).

Due to very limited data available on the occurrence and fate of illicit drugs in the UK it is very difficult to draw any explicit conclusions regarding the possible environmental risk associated with the presence of these compounds in the environment. However, the research undertaken clearly indicates that illicit drugs as emerging contaminants are omnipresent and persistent in the environment. Although they are present in the aquatic environment at low ppt levels, their possible effect on living organisms should not be underestimated. This is because illicit drugs reveal very high pharmacological potency in humans even when administered at very low levels. For example, LSD is among the most potent drugs known, being active in humans at doses from about 20 µg[30]. Its possible potency and toxicity in aquatic organisms is not known. Additionally, illicit drugs usually occur in the environment simultaneously with other pharmacologically active compounds and as a result synergistic action of several active chemicals is to be expected. Communal wastewater and its insufficient treatment are considered to be the main source of environmental contamination. Therefore more research is needed in order to understand the fate of illicit drugs during wastewater treatment and in the environment (both aquatic and terrestrial). In particular, the susceptibility of illicit drugs to biological, chemical and physical processes occurring in the environment, such as microbial degradation, photodegradation, sorption to sludge particles and soil sediments needs to be extensively studied. As several metabolites of illicit drugs are known to be pharmacologically active, studies of their occurrence and fate in the environment are of equal importance. Studies of the possible acute and chronic toxic effects of illicit drugs on aquatic organisms are non-existent and this topic needs urgent attention. The chirality of illicit drugs has to be also considered as it is a major parameter determining the potency and toxicity of drugs. The preliminary research undertaken by Kasprzyk-Hordern et al.[88] aiming at enantioselective analysis amphetamines (amphetamine, methamphetamine, MDEA, MDMA and MDA) during wastewater treatment indicated their non-racemic composition. In the case of methamphetamine, only the more

potent *S*(+)-enantiomer was detected in all treated wastewater samples. The reverse situation was observed in the case of amphetamine, where less potent *R*(-)-enantiomer was present in both raw and treated wastewater at slightly higher concentrations than *S*(+)-enantiomer. The study of enantiomeric composition of MDMA during wastewater treatment indicated its enrichment in one enantiomer only. This might suggest enantioselective processes occurring during treatment, although more comprehensive research has to be undertaken to support such a hypothesis.

5. Conclusions

Pharmacologically active compounds constitute a vast and variable group of chemicals. They are designed to cause a particular pharmacological action in human or veterinary animals/pets. Unfortunately, due to their very often limited metabolism in the body, they are excreted as parent compounds and reach wastewater. Here as a result of very often insufficient wastewater treatment they reach receiving surface waters with treated wastewater or agricultural fields or landfills with sludge. It has to be also remembered that even extensively metabolised pharmaceuticals pose a concern as their metabolites might reveal pharmacological potency or toxicity. Despite low ppt concentrations of these compounds in environmental matrices, pharmacologically active compounds pose considerable environmental concern as many of them are active at very low concentrations. Long-term exposure to these compounds has to be also considered. Additionally, pharmacologically active compounds are present in the environment as a multi-residue mixture of several compounds. Therefore synergistic effects of several compounds should also be considered. Due to the non-volatile nature of the majority of pharmacologically active compounds, and their continuous introduction into the environment, their possible environmental impact cannot be underestimated. Many pharmacologically active compounds are chiral and as a result might reveal different environmental persistence, fate and toxicity, which is enantiomeric ratio dependent. Unfortunately, the phenomenon of chirality, despite its great importance in the pharmaceutical industry has been overlooked by environmental researchers. Currently, environmental fate and toxicity of chiral drugs are assessed without taking into consideration their enantiomeric form. This might lead to a significant under or overestimation of toxicity of chiral drugs and to incorrect environmental risk assessment. Limited research efforts focused on the chirality of drugs in the environment have revealed that the removal of certain chiral drugs such as antidepressant fluoxetine and beta-blocker propranolol during wastewater treatment and their distribution in the aquatic environment are stereoselective. This suggests that certain enantiospecific biological processes, with for example preferential degradation of one enantiomer, take place both during wastewater treatment utilising biological processes and in the environment. It has to be however remembered that changes in enantiomeric fractions of chiral drugs in the environment might also be a consequence of changes in enantioselective processes occurring in humans resulting in non racemic forms of chiral drugs being excreted. Additionally, enantioselective processes are very complex and dependant on the type of organism and chiral compound. As a result studies focused on the enantioselective fate of chiral drugs are very challenging to undertake. This does not only concern interpretation of data obtained but also analysis of chiral drugs as enantioselective analysis of enantiomers of chiral molecules is often problematic.

As discussed above, the study of the enantioselective fate and toxicity of chiral drugs is of great importance and is crucial for a correct risk assessment of the presence of such contaminants in the environment. It has been already proven that the phenomenon of chirality is vital in an assessment of risk posed by chiral pesticides and other environmental pollutants. Extensive research is also needed in the case of chiral drugs, especially those administered as racemates and characterised by different pharmacological potency and/or toxicity of their enantiomers. Research efforts should especially concentrate on a few major groups of chiral drugs. Among them are: NSAIDs, analgesics, CNS drugs (e.g. antidepressants, sedatives, antiepileptics, illicit drugs) and cardiovascular drugs (e.g. beta-blockers) as these drugs are distributed in high quantities all over the world. Many of them are marketed as racemate and often reveal stereoselective potency and metabolism, which might potentially affect their environmental fate and toxicity. Research efforts

should additionally also take into consideration metabolites of chiral drugs as many of them are of high potency, and possibly toxicity towards certain organisms.

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Figures

Fig. 1. Metabolism of warfarin (* - chiral centres; modified from[49]).

Fig. 2. Metabolism of selegiline (modified from [184]).

Fig. 3. Metabolism of thioridazine (modified from[41]).

Fig. 4. Metabolism of omeprazole (modified from[174]).

Fig. 5. Metabolism of ibuprofen through chiral inversion (modified from[49, 37, 56]).

Fig. 6. Introduction of chiral centre as a result of metabolism of achiral drugs (modified from[48, 65, 66]).

Fig. 7. Transformation of chiral drugs in the environment

Fig. 8. Structures of chiral NSAIDs, analgesics and anaesthetics.

Fig. 9. Major oxidative metabolic pathways of ibuprofen (modified from[185]).

Fig. 10. Environmental occurrence of chiral drugs – NSAIDs and analgesics (maximum (♦) and mean (□) concentrations)[5, 8, 9, 18, 102-105, 107, 110, 111, 154, 166-169, 186-198].

Fig. 11. Structures of chiral CNS drugs.

Fig. 12. Metabolism of citalopram (modified from[41]).

Fig. 13. Metabolism of trimipramine (modified from[41]).

Fig. 14. Environmental occurrence of chiral drugs – CNS drugs (maximum (♦) and mean (□) concentrations)[8, 9, 25, 106, 116, 120, 125, 130-132, 146, 148, 169, 176, 186, 188, 189, 192, 199-204].

Fig. 15. Structures of chiral cardiovascular drugs.

Fig. 16. Stereoselective metabolism of propranolol (modified from[150]).

Fig. 17. Environmental occurrence of chiral drugs – cardiovascular drugs (maximum (♦) and mean (□) concentrations)[8, 9, 15, 18, 116, 130, 148, 152, 167, 186, 187, 192, 199, 203-207].

Fig. 18. Metabolism of verapamil (modified from[157]).

Fig. 19. Metabolism of flecainide (modified from[157]).

Fig. 20. Structures of other chiral drugs.

Fig. 21. Metabolism of pantoprazole (modified from[92])

Fig. 22. Environmental occurrence of chiral drugs – illicit drugs (maximum (♦) and mean (□) concentrations)[5-9, 21-28, 146, 198, 208-210].

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Fig. 1

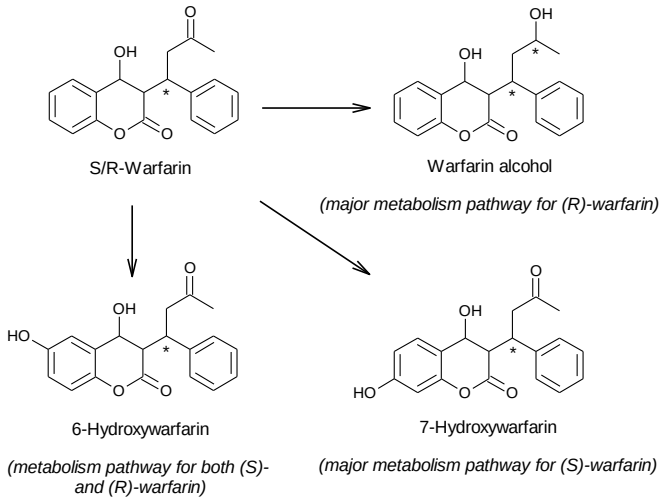

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Fig. 2

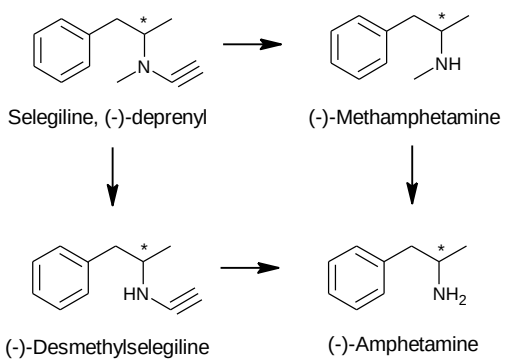

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Fig. 3

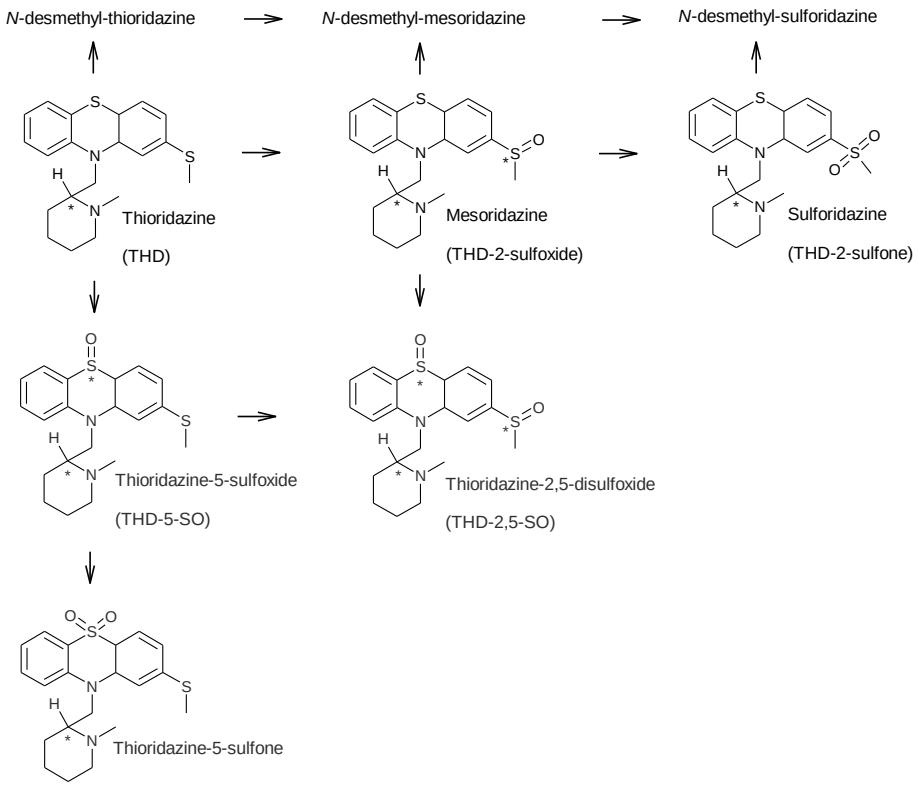

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Fig. 4

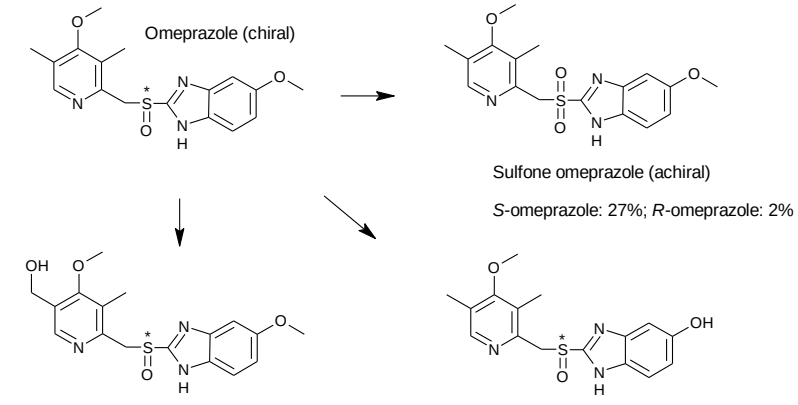

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Fig. 5

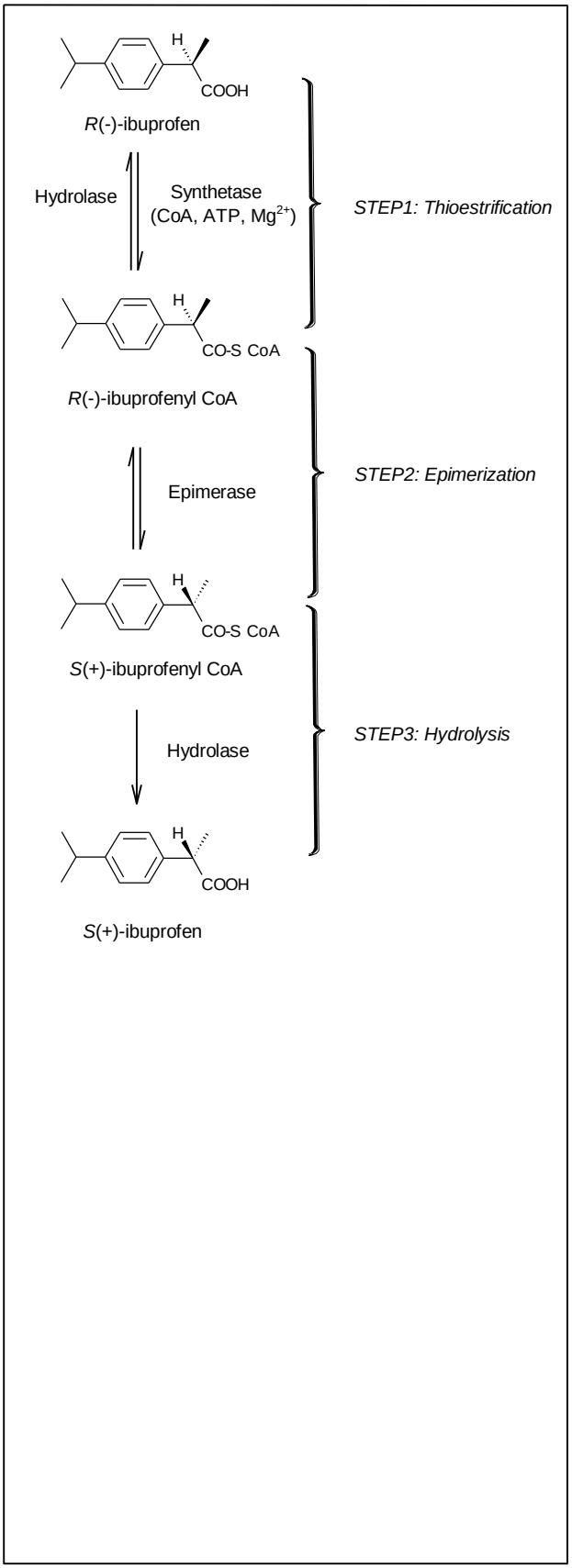

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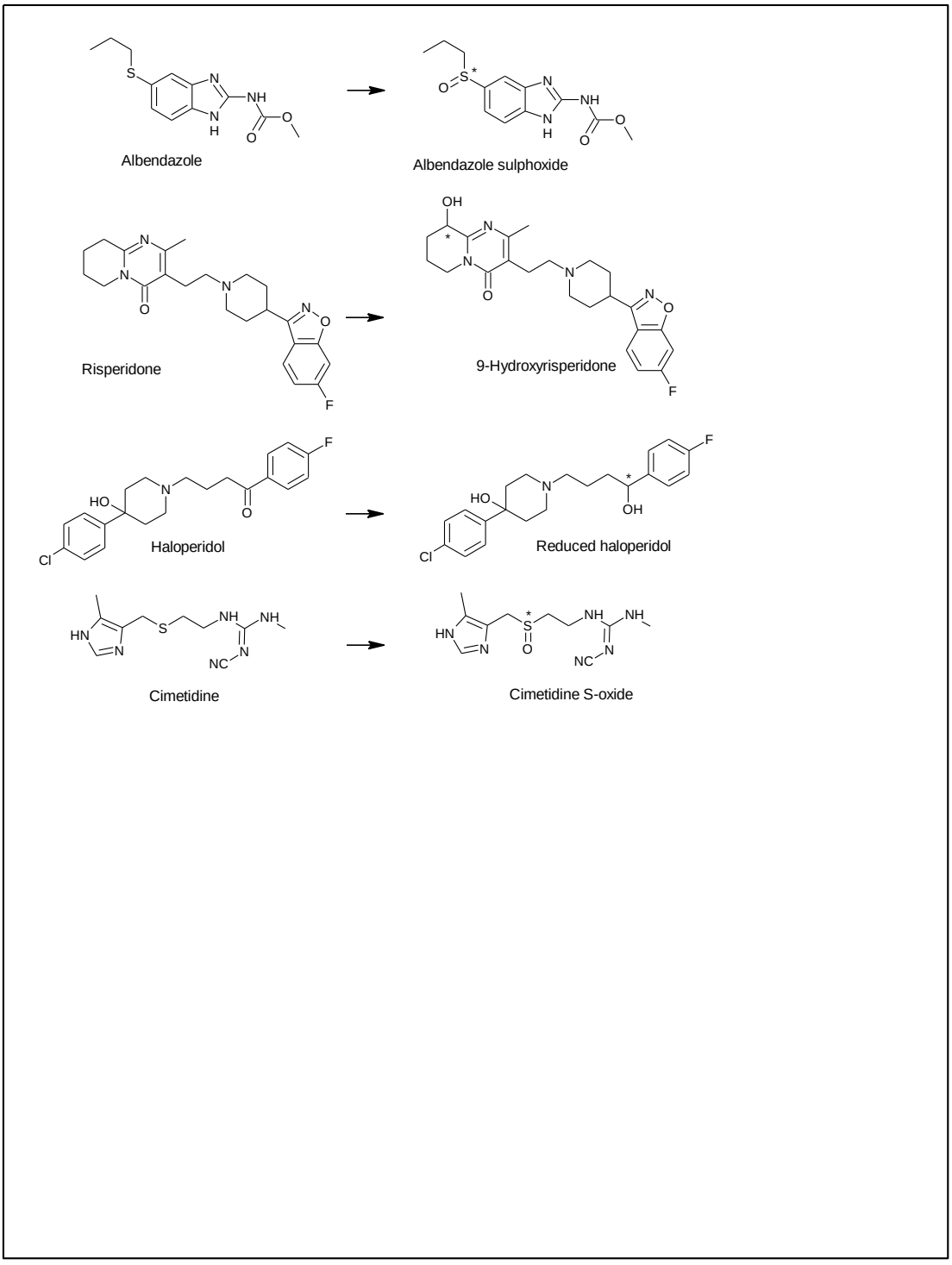
Fig. 6



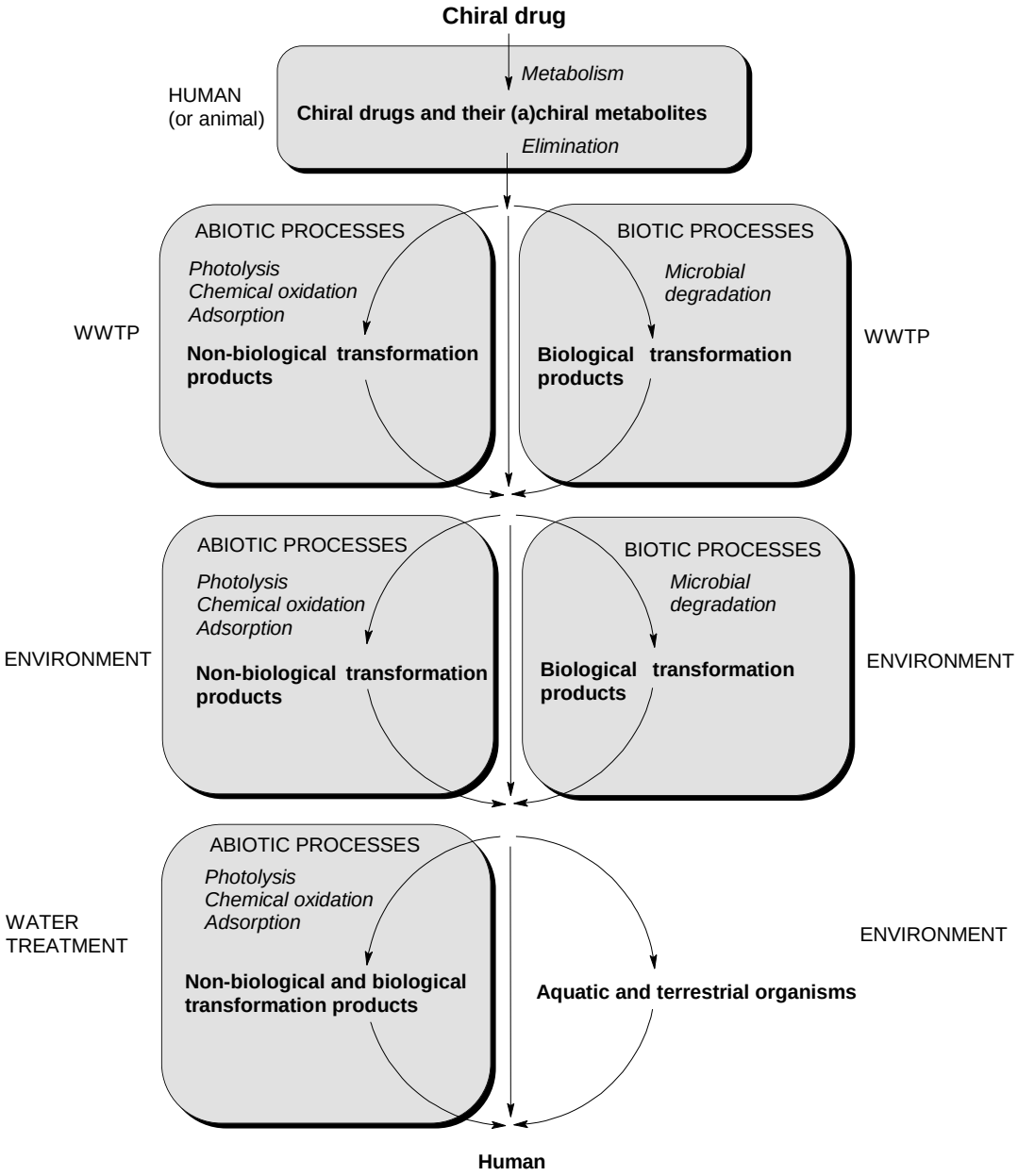
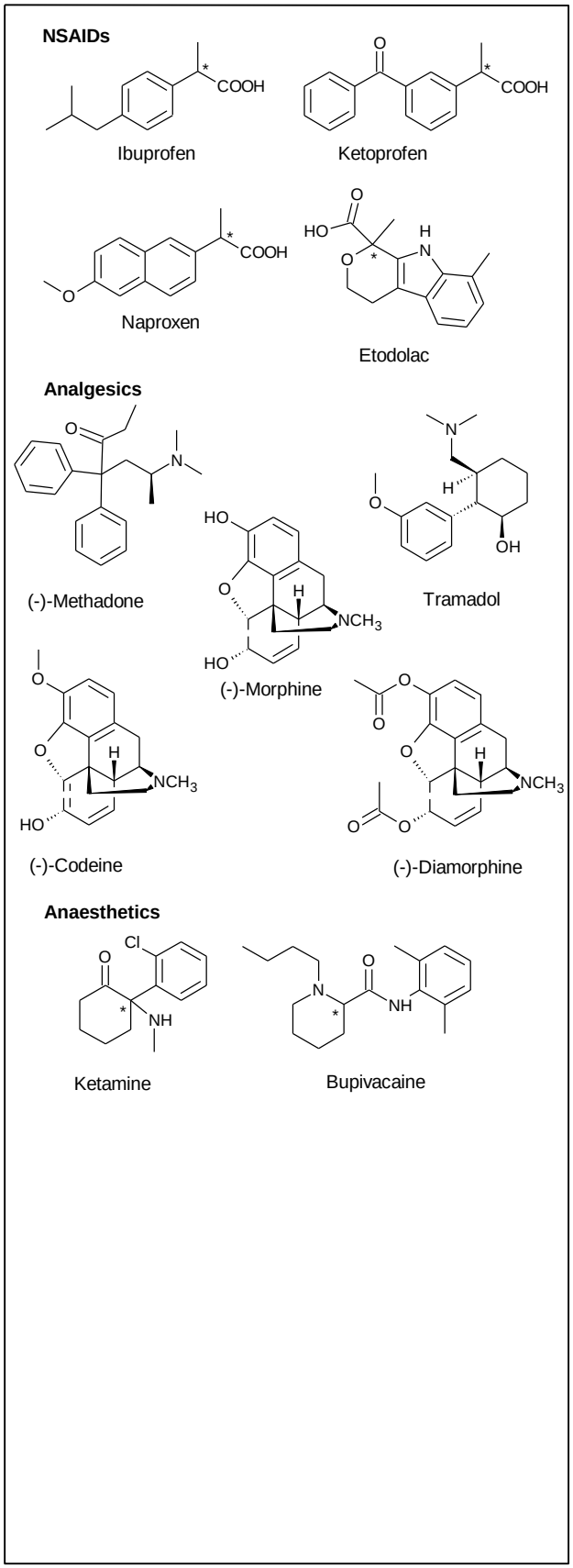

Chiral drug
HUMAN
(or animal)
Metabolism
Chiral drugs and their (a)chiral metabolites
Elimination
ABIOTIC PROCESSES
Photolysis
Chemical oxidation
Adsorption
Non-biological transformation products
BIOTIC PROCESSES
Microbial degradation
Biological transformation products
WWTP
WWTP
ENVIRONMENT
ENVIRONMENT
WATER TREATMENT
ENVIRONMENT
Non-biological and biological transformation products
Aquatic and terrestrial organisms
Human

Fig. 7

All text and images must be placed within the frame.

Fig. 8



All text and images must be placed within the frame.

Fig. 9

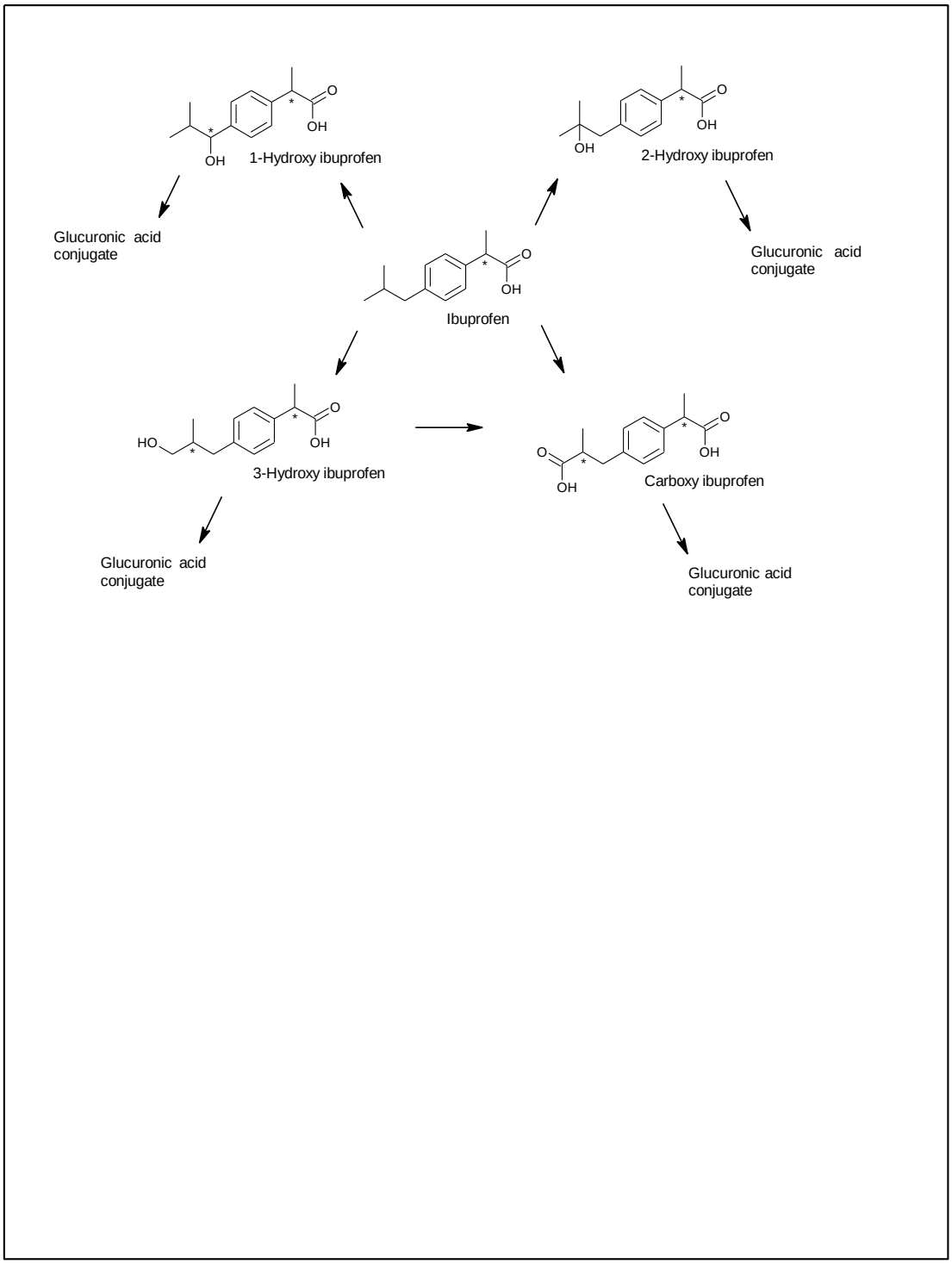
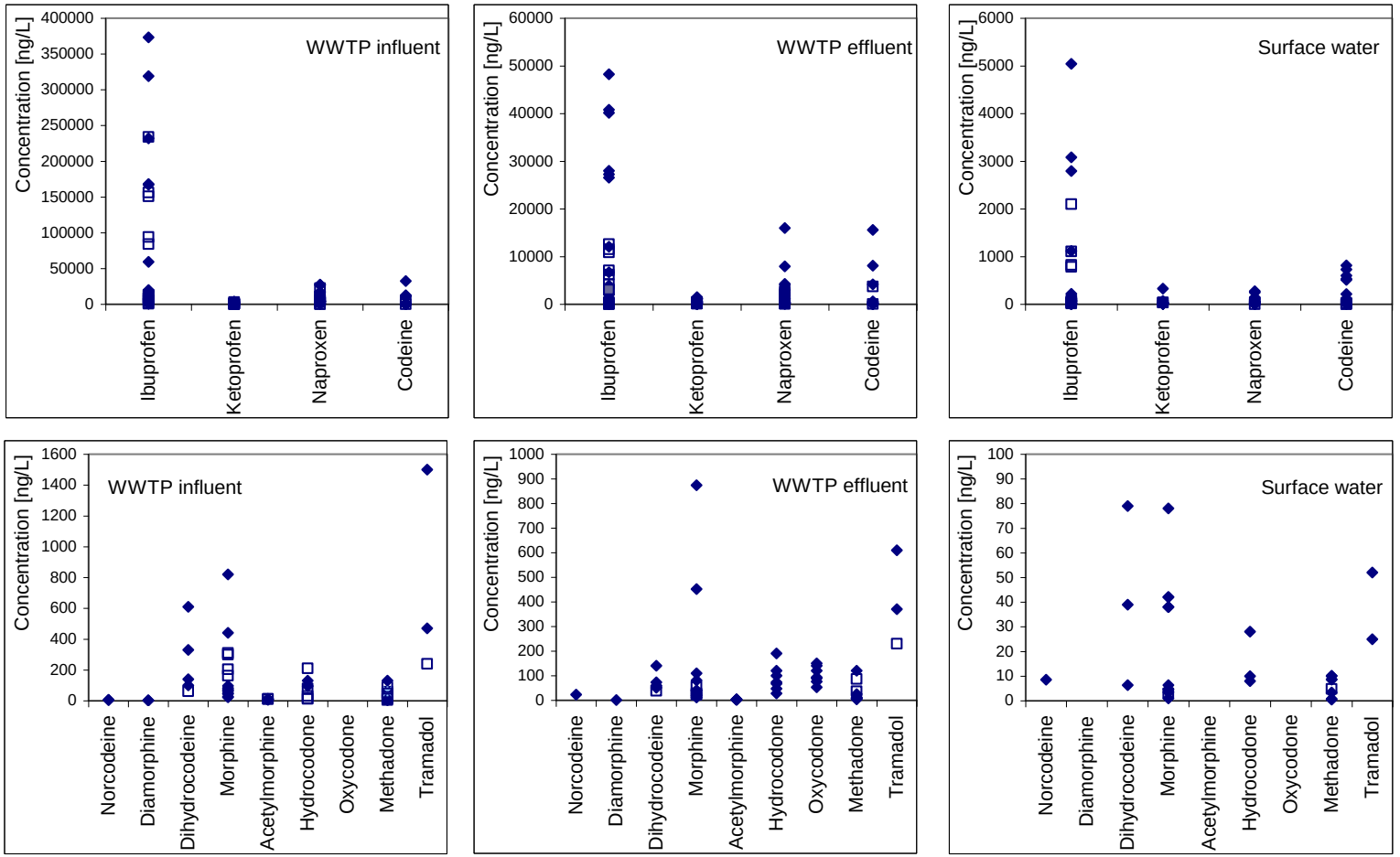
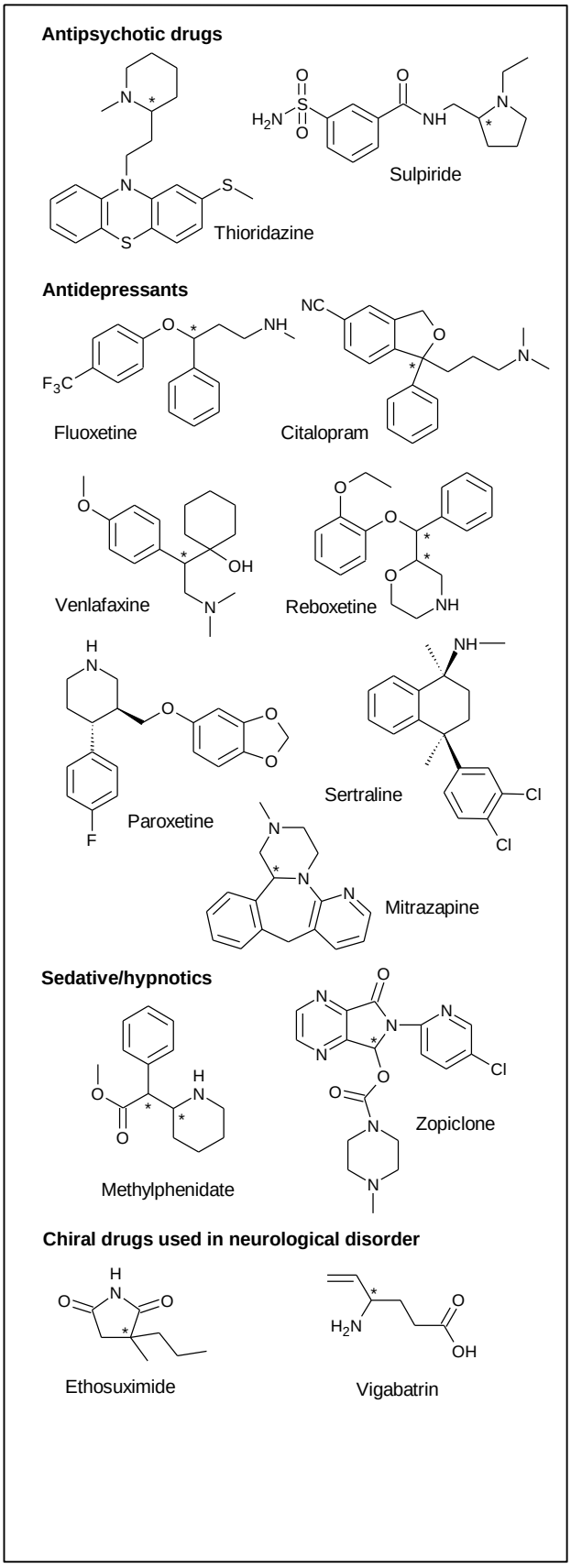


Fig. 10


WWTP influent
WWTP effluent
Surface water
Concentration [ng/L]
Ibuprofen
Ketoprofen
Naproxen
Codeine
Norcodeine
Diamorphine
Dihydrocodeine
Morphine
Acetylmorphine
Hydrocodone
Oxycodone
Methadone
Tramadol

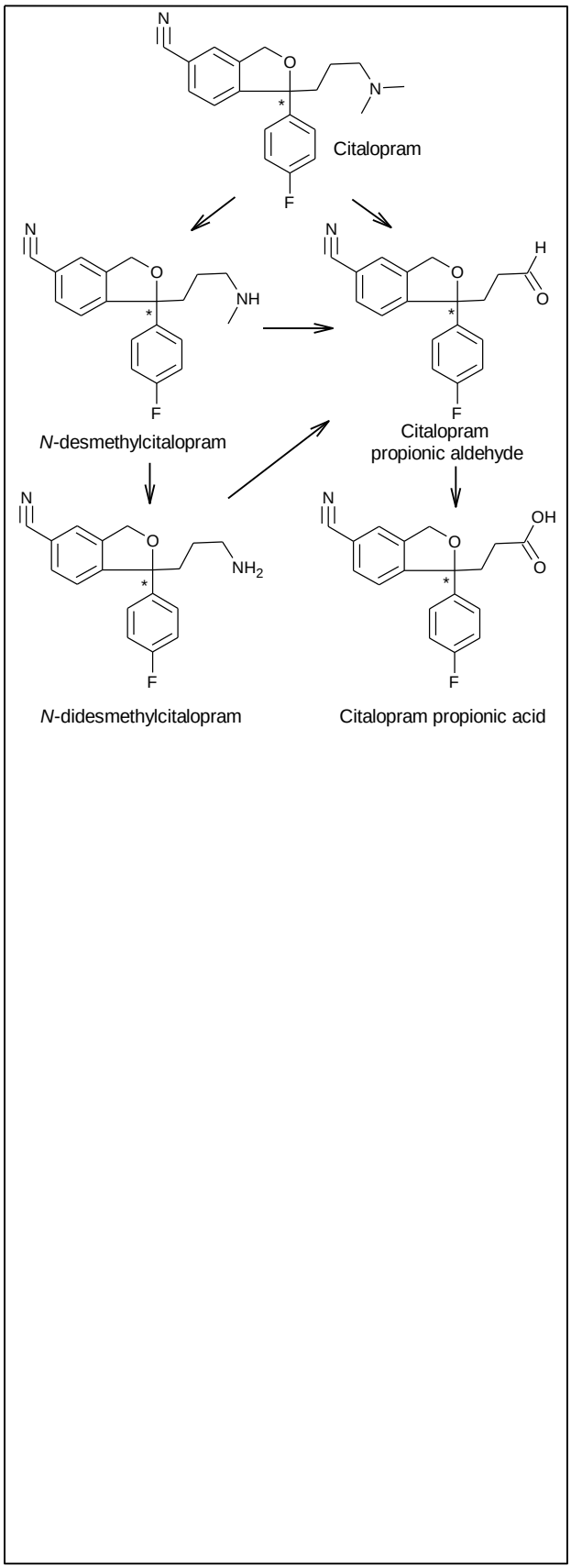
All text and images must be placed within the frame.

Fig. 11



All text and images must be placed within the frame.

Fig. 12



All text and images must be placed within the frame.

Fig. 13

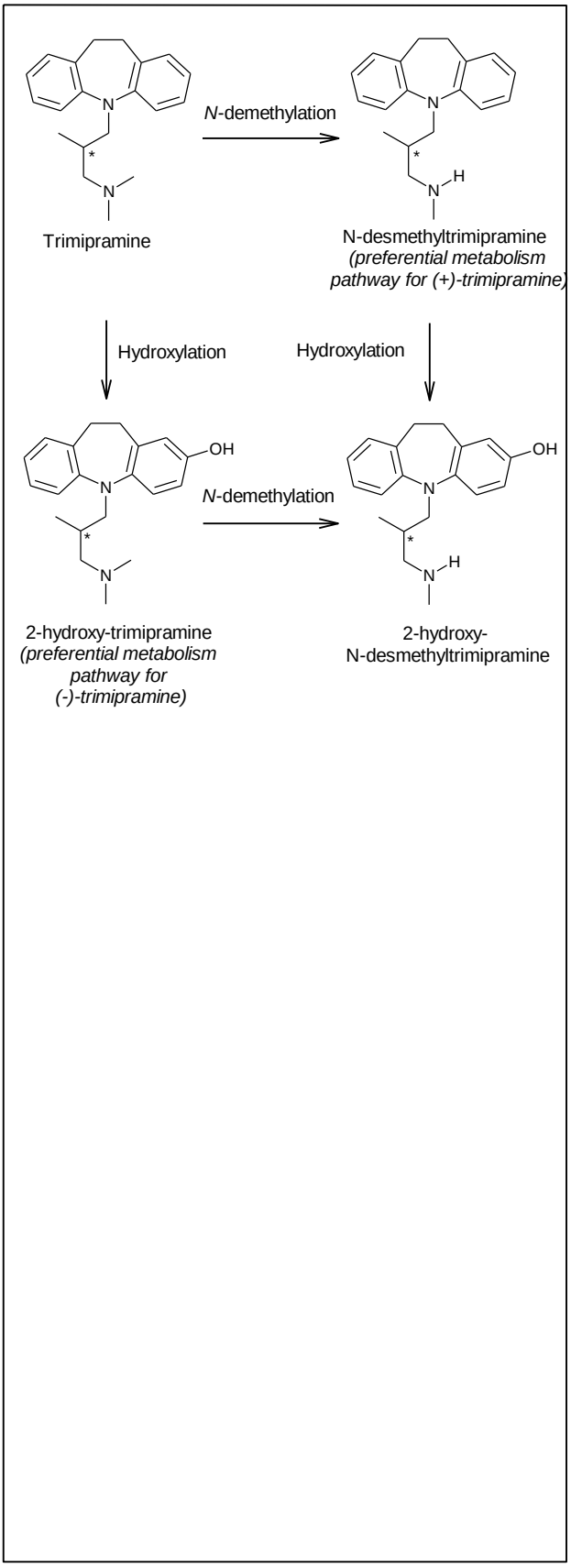
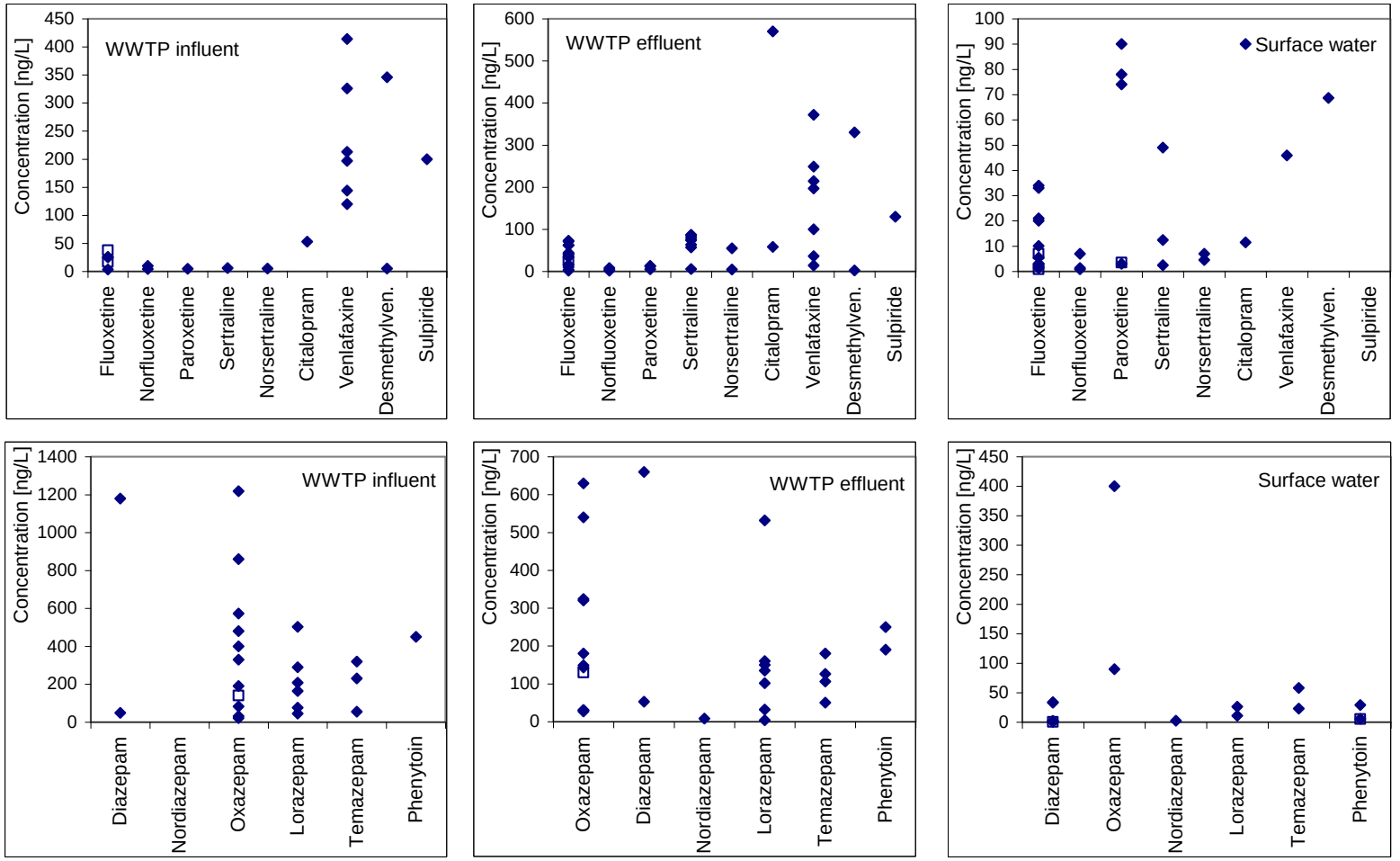
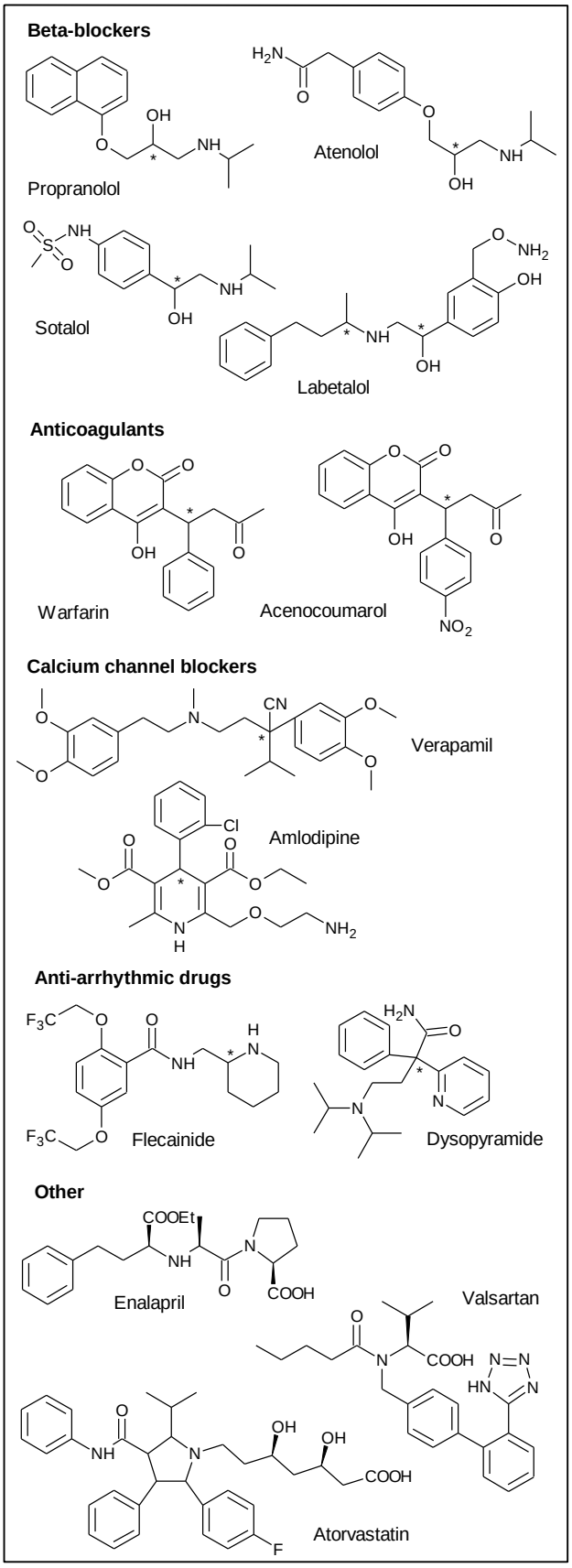


Fig. 14.


WWTP influent
Concentration [ng/L]
0
50
100
150
200
250
300
350
400
450
Fluoxetine
Norfluoxetine
Paroxetine
Sertraline
Norsertraline
Citalopram
Venlafaxine
Desmethylven.
Sulpiride
WWTP effluent
Concentration [ng/L]
0
100
200
300
400
500
600
Fluoxetine
Norfluoxetine
Paroxetine
Sertraline
Norsertraline
Citalopram
Venlafaxine
Desmethylven.
Sulpiride
Surface water
Concentration [ng/L]
0
10
20
30
40
50
60
70
80
90
100
Fluoxetine
Norfluoxetine
Paroxetine
Sertraline
Norsertraline
Citalopram
Venlafaxine
Desmethylven.
Sulpiride
WWTP influent
Concentration [ng/L]
0
200
400
600
800
1000
1200
1400
Diazepam
Nordiazepam
Oxazepam
Lorazepam
Temazepam
Phenytoin
WWTP effluent
Concentration [ng/L]
0
100
200
300
400
500
600
700
Oxazepam
Diazepam
Nordiazepam
Lorazepam
Temazepam
Phenytoin
Surface water
Concentration [ng/L]
0
50
100
150
200
250
300
350
400
450
Diazepam
Oxazepam
Nordiazepam
Lorazepam
Temazepam
Phenytoin

All text and images must be placed within the frame.

Fig. 15



All text and images must be placed within the frame.

Fig. 16

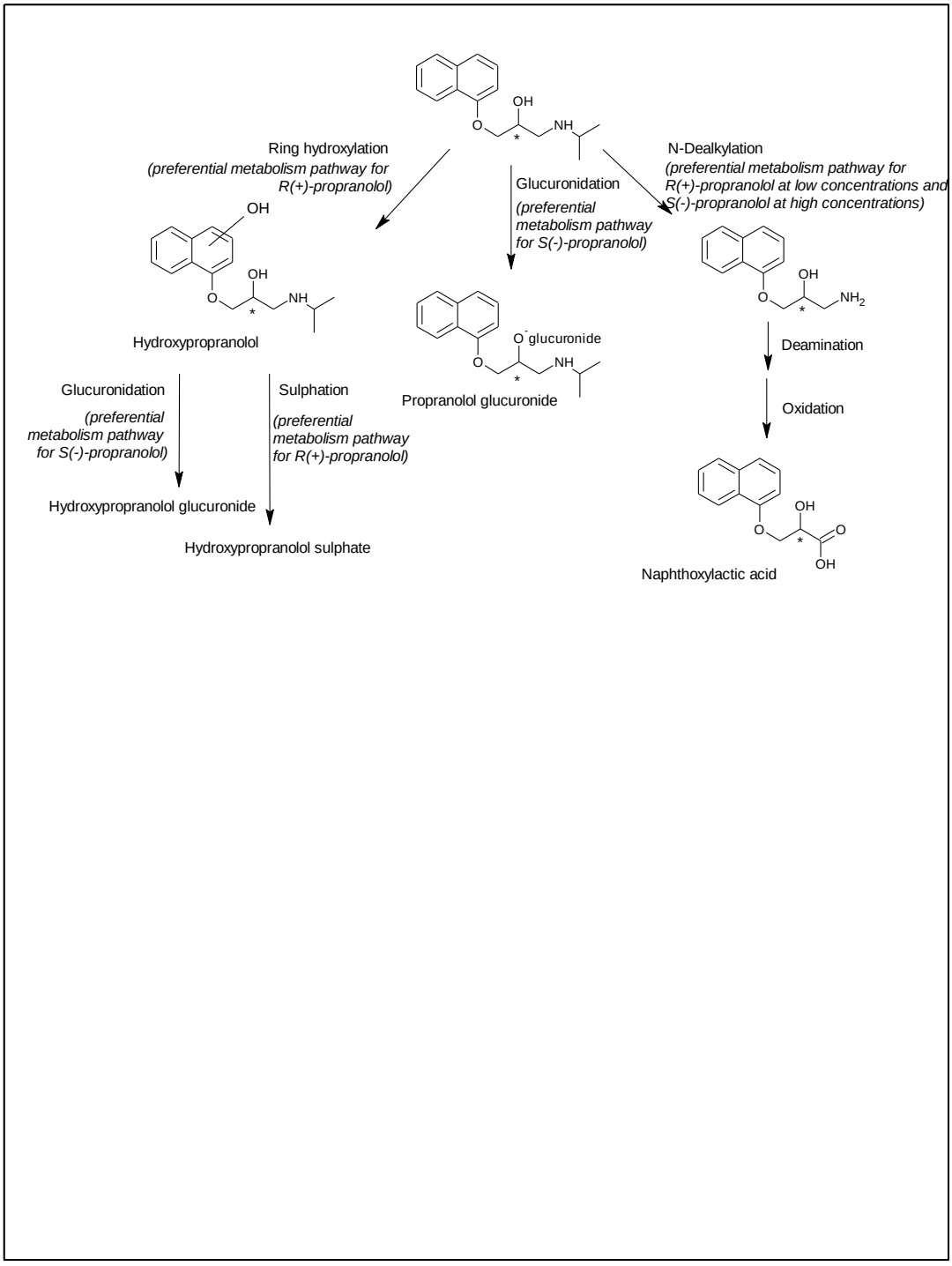
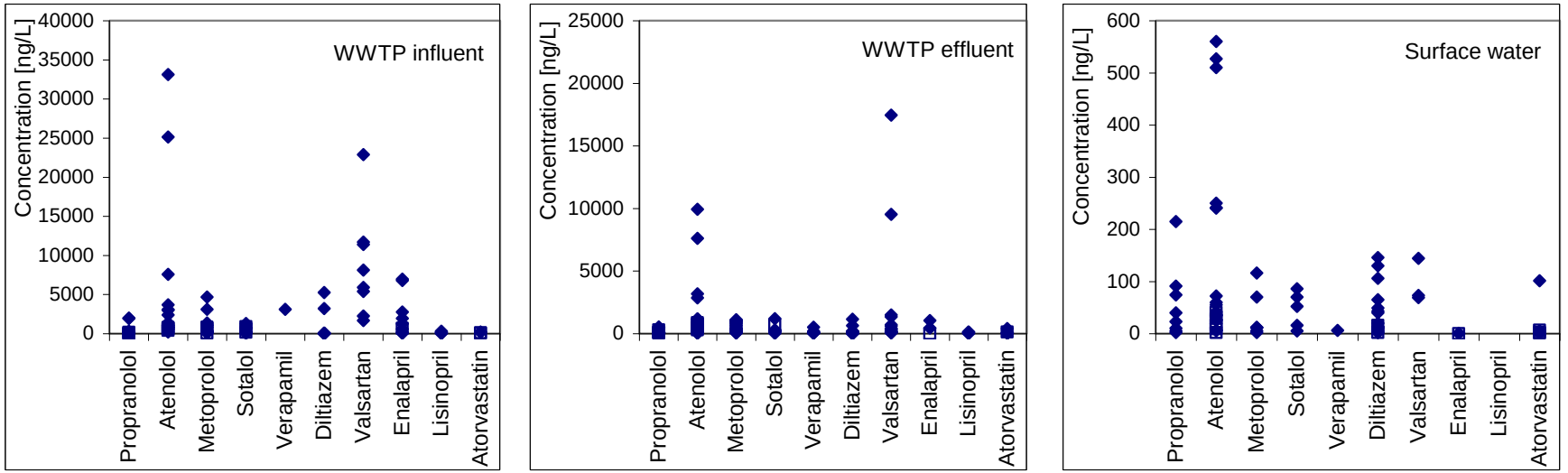
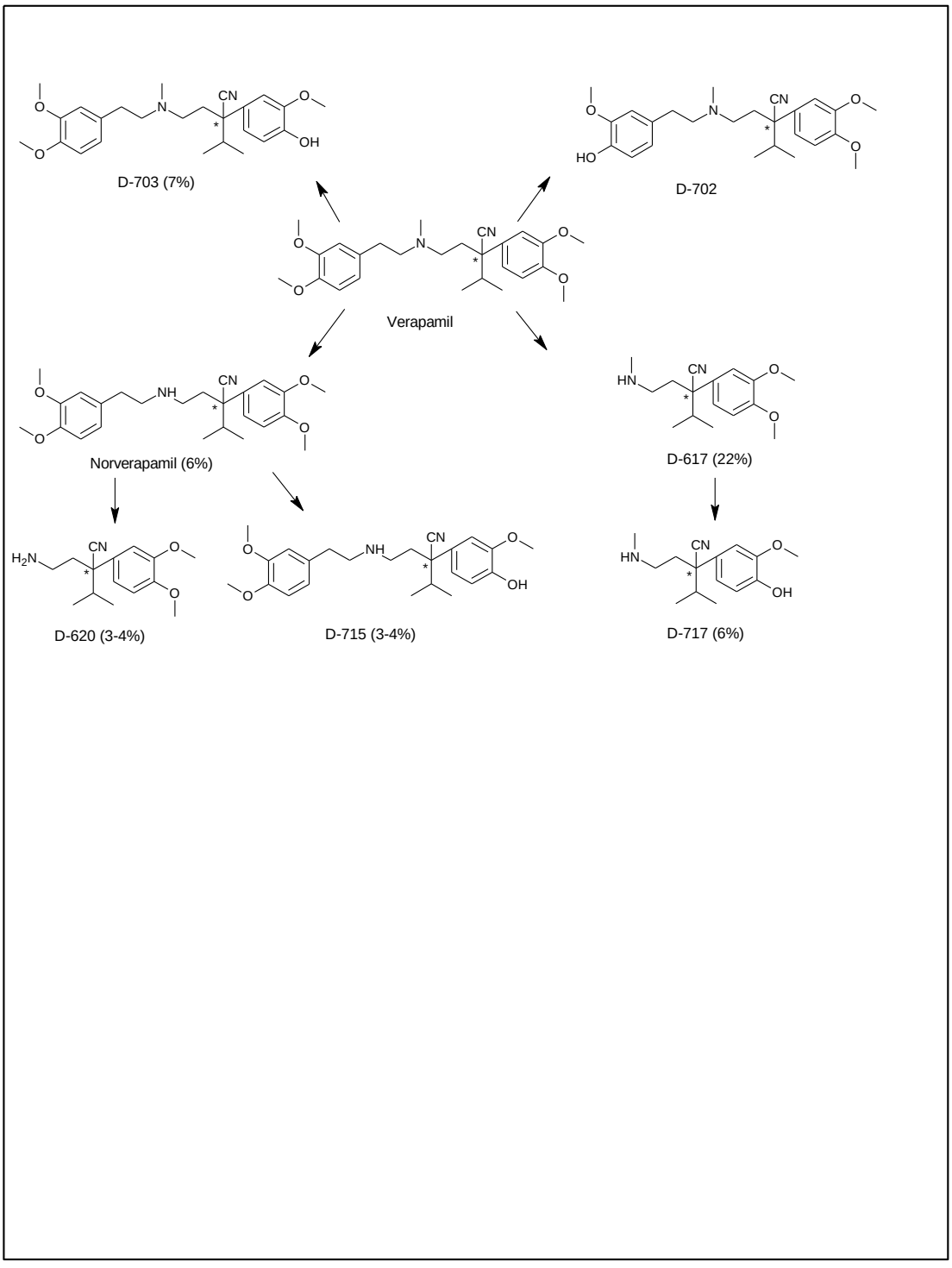


Fig.17


WWTP influent
Concentration [ng/L]
40000
35000
30000
25000
20000
15000
10000
5000
0
Propranolol
Atenolol
Metoprolol
Sotalol
Verapamil
Diltiazem
Valsartan
Enalapril
Lisinopril
Atorvastatin
WWTP effluent
Concentration [ng/L]
25000
20000
15000
10000
5000
0
Propranolol
Atenolol
Metoprolol
Sotalol
Verapamil
Diltiazem
Valsartan
Enalapril
Lisinopril
Atorvastatin
Surface water
Concentration [ng/L]
600
500
400
300
200
100
0
Propranolol
Atenolol
Metoprolol
Sotalol
Verapamil
Diltiazem
Valsartan
Enalapril
Lisinopril
Atorvastatin

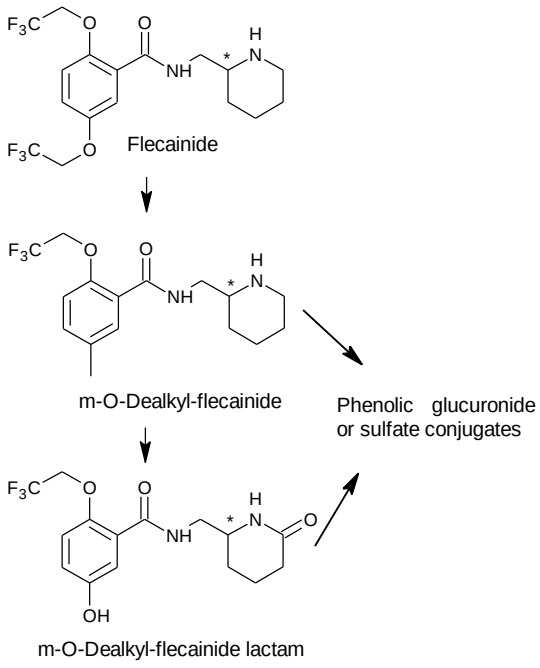
All text and images must be placed within the frame.

Fig. 18



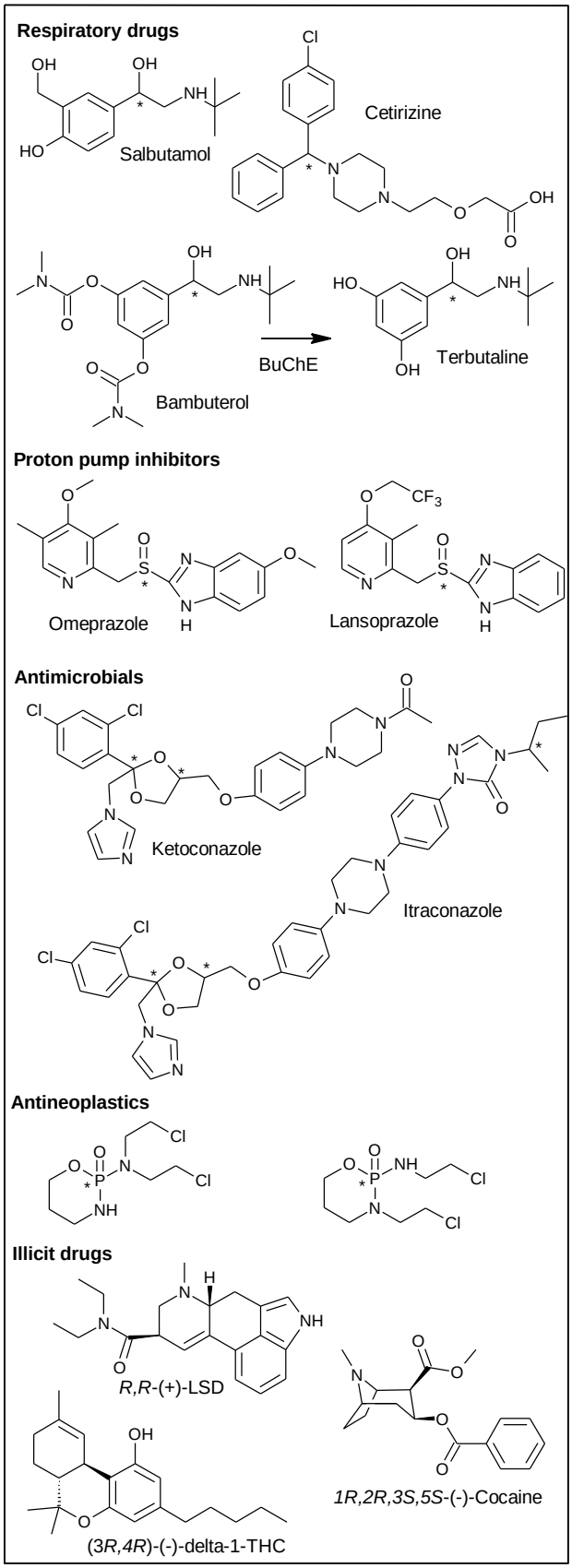
All text and images must be placed within the frame.

Fig. 19



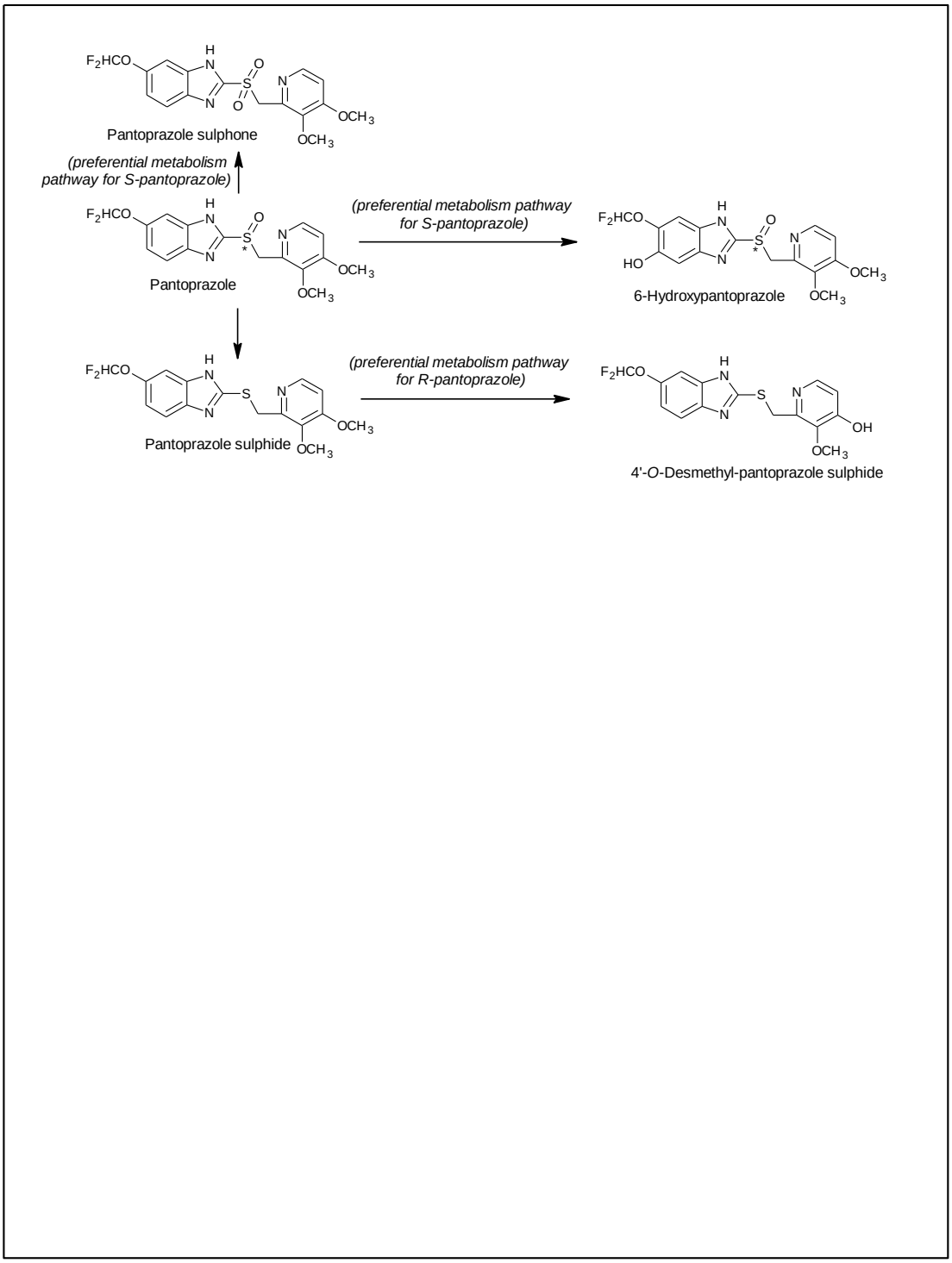
All text and images must be placed within the frame.

Fig. 20



All text and images must be placed within the frame.

Fig. 21



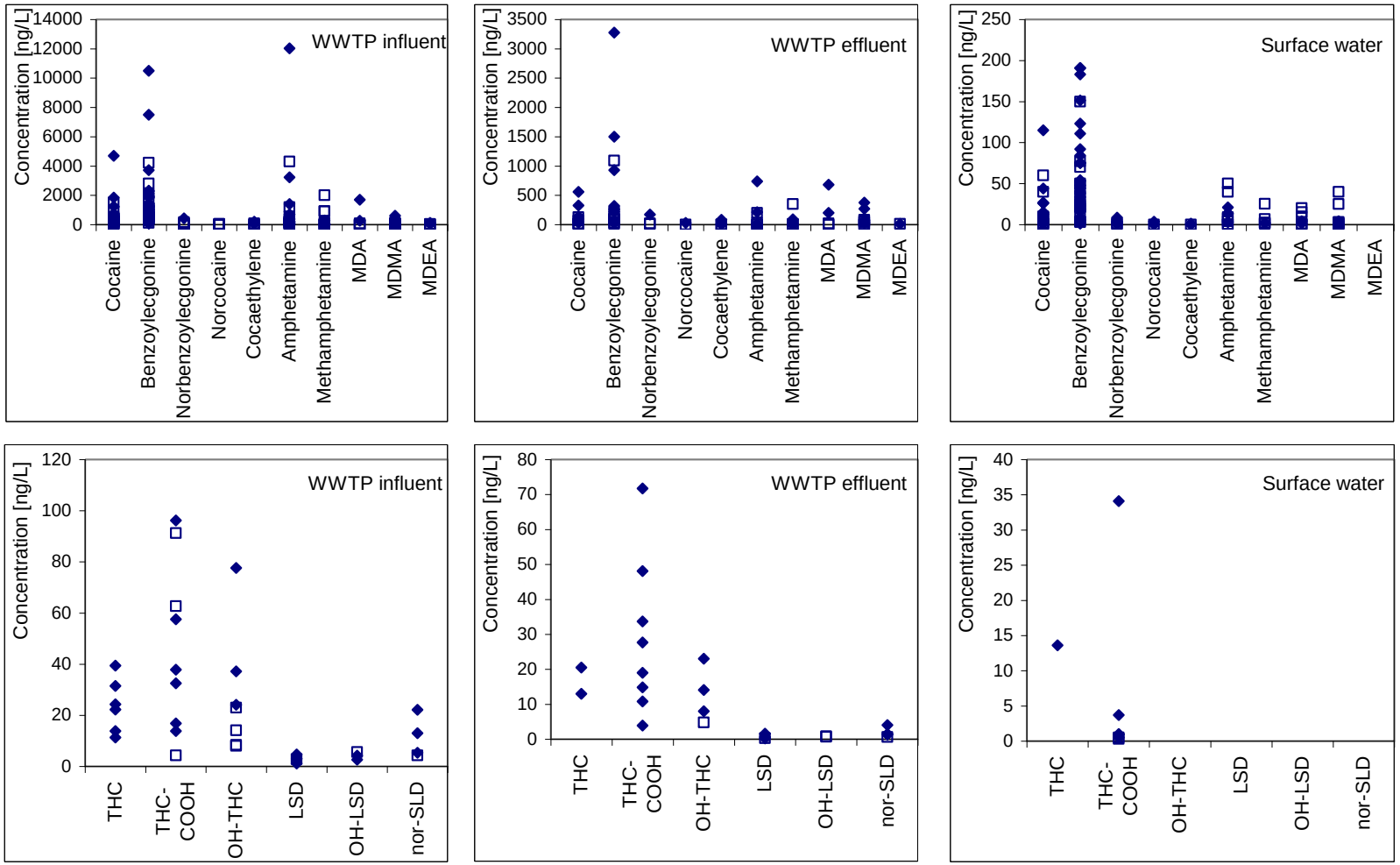

WWTP influent
WWTP effluent
Surface water
Concentration [ng/L]
Cocaine
Benzoylecgonine
Norbenzoylecgonine
Norcocaine
Cocaethylene
Amphetamine
Methamphetamine
MDA
MDMA
MDEA
THC
THC-COOH
OH-THC
LSD
OH-LSD
nor-SLD

Fig. 22

Table 1. New synthetic drugs launched in the world in years 1985-2004[43].

Year	Racemates [%]	Single enantiomers [%]	Achiral [%]
1983	37	26	37
1984	28	26	46
1985	38	22	40
1986	26	26	48
1987	18	49	33
1988	26	39	35
1989	29	26	45
1990	33	35	32
1991	20	40	40
1992	21	44	35
1993	16	45	39
1994	38	38	24
1995	21	46	33
1996	9	41	50
1997	24	30	46
1998	15	50	35
1999	13	52	35
2000	9	62	29
2001	0	68	32
2002	6	55	39

Table. 2. Racemic switches for chiral drugs[38, 39, 43, 46, 122].

Chiral drug (racemate)	**Enantiomer**	**Action of single enantiomer**	**Status**
Fenfluramine	Dexfenfluramine *S*(+)-Fenfluramine	Anorectic	Launched as single enantiomer in US (1996); withdrawn in 1997
Ofloxacin	Levofloxacin *S*(-)-Ofloxacin	Antibacterial	Sold as single enantiomer in JP (1995)
Labetalol	Dilevalol	Beta-blocker	Withdrawn
Ibuprofen	Dexibuprofen *S*(+)-Ibuprofen	Anti-inflammatory	Launched as single enantiomer in Austria (1994)
Ketoprofen	Dexketoprofen *S*(+)-Dexketoprofen	Anti-inflammatory	Launched as single enantiomer in EU (1998)
Bupivacaine	Levobupivacaine *S*(-)-Bupivacaine	Local anaesthetic	Launched as single enantiomer in US (2000)
Ketamine	*S*-Ketamine	Anaesthetic	Sold as single enantiomer in Germany
Fluoxetine	*S*-Fluoxetine	Antidepressant	Development stopped
Omeprazole	Esomeprazole *S*(-)-Omeprazole	Proton-pump inhibitor	Launched as single enantiomer in EU (2000)
Salbutamol (albuterol)	*R*(-)-Salbutamol (*R*(-)-Albuterol)	Anti-asthmatic	Launched as single enantiomer in the US (1999)
Cetirizine	Levocetirizine *R*(-)-Cetrizine	Allergy, antihistamine	Launched as single enantiomer in EU (2001)
Methylphenidate ((*R*,*R*)(+), (*S*,*S*)(-))	Dexmethylphenidate (*R*,*R*)(+)-Methylphenidate	Attention-deficit hyperactivity disorder	Launched as single enantiomer in US (2001)
Citalopram	Escitalopram *S*(+)-citalopram	SSRI	Launched as single enantiomer in EU (2001)
Zopiclone	Eszopiclone	Hipnotic	Launched as single enantiomer (2004)

Table 3. Enantioselective analysis of chiral drugs in environmental matrix.

Chiral drugs	Resolution	Chromatographic conditions	Reference
Atenolol Metoprolol Propranolol	0.8 0.7 0.9	Direct chiral LC/MS/MS Sample preparation: SPE (Oasis HLB); LC/MS/MS: Chirobiotic V vancomycin-based chiral column; mobile phase: 90/10 H_2O/MeOH, TEA, CH_3COOH (pH=4)	79
Atenolol Metoprolol Propranolol Pindolol Nadolol Sotalol Citalopram Fluoxetine B_2-agonist: Sabutamol	1.15 1.10 1.32 0.99 0.70 1.34 1.06 1.88 0.98	Direct chiral LC/MS/MS Sample preparation: SPE (Oasis HLB); LC/MS/MS: Chirobiotic V vancomycin-based chiral column; mobile phase: 90/10 H_2O/MeOH, TEA, NH_4OAc, HCOOH (pH=4) MQL, 1-24 ng L^{-1} (wastewater)	84
Propranolol Metoprolol	-	Indirect chiral GC/MS/MS Sample preparation: SPE (C18); derivatisation with MSTFA and (-)-MPTA-Cl LOD, 0.1-1 ng L^{-1} (surface water and wastewater)	83
Ibuprofen Naproxen	-	Direct chiral GC/MS Sample preparation: SPE (Strata X) GC/MS: Astec Chiraldex dimethyl β-cyclodextrin chiral column	85
Ibuprofen Carboxy-ibuprofen Hydroxy-ibuprofen	-	Direct chiral GC/MS Sample preparation: SPE (Bio-Beads SM-2, polystyrene divinylbenzene copolymer) GC/MS: OV1701-DMPen (DMPen = heptakis(2,6,-O-dimethyl-3-O-n-pentyl)-β-cyclodextrin)	90
Amphetamine Methamphetamine MDEA MDMA MDA Ephedrine/Pseudoephedrine Norephedrine Venlafaxine	2.2 1.2 1.2 3.2 4.0 3.6 1.1 1.1	Direct chiral LC/MS/MS Sample preparation: SPE (Oasis HLB); LC/MS/MS: Chiral CBH column; mobile phase: 90/10 H_2O/2-propanol, 1mM ammonium acetate (pH=5)	88

Table 4. Enantiomer fractions (*EF*) of chiral drugs and enantiomer enrichment during wastewater treatment

Drug	WWTP: treatment	Wastewater influent			Wastewater effluent			Reference
		Conc [ng L^{-1}]	*EF*		Conc [ng L^{-1}]	*EF*		
Propranolol	WWTP1 (Aug): biological, UV	~100	~0.47[1]	Racemic	~100	~0.37[1]	S(-)>R(+)	79
	WWTP1 (Nov): biological, UV	0	-	-	~20	~0.45[1]	Racemic	
	WWTP2 (Sep): aeration	~10	-	-	~5	~0.38[1]	S(-)>R(+)	
Atenolol	WWTP1 (Aug): biological, UV	~650	~0.48[1]	Racemic	~780	~0.42[1]	S(-)>R(+)	
	WWTP1 (Nov): biological, UV	~1100	~0.49[1]	Racemic	~600	~0.48[1]	Racemic	
	WWTP2 (Sep): aeration	~800	~0.39[1]	S(-)>R(+)	~180	~0.46[1]	Racemic	
Metoprolol	WWTP1 (Aug): biological, UV	~550	~0.41[2]	Racemic	~400	~0.53[2]	Racemic	
	WWTP1 (Nov): biological, UV	~400	~0.54[2]	Racemic	~400	~0.5[2]	Racemic	
	WWTP2 (Sep): aeration	~310	~0.60[2]	E1>E2	~180	~0.45[2]	E1<E2	
Atenolol	WWTP1: biological, UV	971	~0.53[1]	R(+)>S(-)	664	~0.50[1]	Racemic	84
Propranolol		10	~0.5[1]	Racemic	45	~0.42[1]	S(+)<R(-)	
Fluoxetine		18	~0.2[1]	S(+)<R(-)	14	~0.3[1]	S(+)<R(-)	
Metoprolol		411	~0.52[2]	E1>E2	375	~0.52[2]	E1>E2	
Nadolol		51	~0.69[2]	E1>E2	20	~0.79[2]	E1>E2	
Sotalol		529	~0.56[2]	E1>E2	466	~0.56[2]	E1>E2	
Citalopram		307	~0.57[2]	E1>E2	207	~0.63[2]	E1>E2	
Sabutamol		20	~0.39[2]	E1<E2	17	~0.40[2]	E1<E2	
Propranolol	WWTP1 (Apr): activated sludge	23	0.50[1]	R(+)=S(-)	13	0.44[1]	R(+)<S(-)	83
	WWTP1 (Sep): activated sludge	13	0.50[1]	R(+)=S(-)	11	0.42[1]	R(+)<S(-)	
	WWTP2 (Apr): activated sludge	250	0.49[1]	R(+)=S(-)	58	0.41[1]	R(+)<S(-)	
	WWTP2 (Sep): activated sludge	-	-	-	21	0.40[1]	R(+)<S(-)	
	WWTP3 (May): trickling filter	58	0.54[1]	R(+)>S(-)	3	0.33[1]	R(+)<S(-)	
	WWTP4 (Jun): activated sludge	-	-	-	10	0.37[1]	R(+)<S(-)	
	WWTP5 (Jun): activated sludge	-	-	-	9	0.30[1]	R(+)<S(-)	
	WWTP6 (Jun): activated sludge	22	0.52[1]	R(+)>S(-)	53	0.33[1]	R(+)<S(-)	
	WWTP7 (Jul): activated sludge	-	-		160	0.31[1]	R(+)<S(-)	
Ibuprofen	WWTP1: activated sludge		0.88[3]	S>R		0.64[3]	S>R	85
Naproxen			0.88[3]	S>R		0.86[3]	S>R	
Ibuprofen	WWTP1 (Oct)	3300	6.2[4]	S>R	~2	~1.5[4]	S>R	90
	WWTP1 (Nov)	990	5.7[4]	S>R	-	-		
	WWTP1 (Dec)	2900	8.0[4]	S>R	~2	~2[4]	S>R	
	WWTP2 (Feb)	1360	7.9[4]	S>R	13	0.9[4]	S<R	
	WWTP3 (Feb)	2040	5.5[4]	S>R	81	1.0[4]	S=R	
Amphetamine	WWTP1	368.1	0.58[2]	R(-)>S(+)	-	-	-	88
	WWTP2	63.7	0.62[2]	R(-)>S(+)	-	-	-	
	WWTP3	73.6	0.54[2]	R(-)>S(+)	-	-	-	
	WWTP4	181.7	0.68[2]	R(-)>S(+)	-	-	-	
Venlafaxine	WWTP1	226.3	0.50[2]	E1=E2	265.6	0.43[2]	E1<E2	
	WWTP2	630.3	0.45[2]	E1<E2	426.5	0.42[2]	E1<E2	
	WWTP3	156.5	0.46	E1<E2	239.9	0.48[2]	E1<E2	
	WWTP4	113.9	0.50	E1=E2	217.2	0.37[2]	E1<E2	

[1] - EF = (+)/[(+)+(-)] where (+) and (-) are peak areas of the (+) and (-) enantiomers

[2] - $EF = E1/[E1+E2]$ where *E1* and *E2* are peak areas of the first and second-eluted enantiomers

[3] - $EF=S/(R/S+R)$ where *S* and *R* are peak areas of the *S* and *R* enantiomers

[4] - $ER=S/R$ where *S* and *R* are peak areas of the *S* and *R* enantiomers

Table 5. Enantiomer fractions (*EF*) of chiral drugs in surface water.

Drug	River	Conc [ng L^{-1}]	*EF*		Reference
Metoprolol	Trinity River, Texas (travel time, ~0 days)	~390	~0.45[1]		82
	~4	~340	~0.44[1]		
	~8	~180	~0.41[1]		
	~11	~90	~0.38[1]		
	~13.5	~40	~0.31[1]		
Ibuprofen	Greifenesee (outlet) (Aug)	4.3	~0.7[2]	S<R	90
	Greifenesee (outlet) (Sep)	4.7	~1.0[2]	S=R	
	Greifenesee (outlet) (Dec)	7.8	2.0[2]	S>R	
	Greifenesee (outlet) (Mar)	4.3	2.1[2]	S>R	
	Greifenesee (outlet) (Apr)	7.8	2.0[2]	S>R	
	Greifenesee (outlet) (May)	2.0	1.6[2]	S>R	
	Greifenesee (outlet) (Jul)	5.2	1.6[2]	S>R	
	Greifenesee (outlet) (Aug)	5.2	1.1[2]	S>R	
	Greifenesee (outlet) (Dec)	4.7	1.8[2]	S>R	
	Aabach Tributary (Aug-Oct)	<0.2-2.4	0.9-3.0[2]	S<R-S>R	
	Pfäffikersee (Aug)	4.0	1.4[2]	S>R	
	Zürichsee (Dec-Oct)	3.3-4.0	1.0[2]	S=R	
	Baldeggrersee (Jun-Nov)	1.5-3.2	1.3[2]	S>R	
	Sempachersee (Aug-Jul)	<0.2	1.8-4.1[2]	S>R	

[1] *EF* = (+)/[(+)+(-)] where (+) and (-) are peak areas of the (+) and (-) enantiomers

[2] *ER*=*S*/*R* where *S* and *R* are peak areas of the *S* and *R* enantiomers

Table 6. Prescription of chiral drugs in England – selected chiral NSAIDS and analgesics (approximate values)[19].

Chiral drug	Marketed as	Prescription [tonnes year^{-1}]				
		2004	2005	2006	2007	2008
NSAIDs:						
Etodolac	Racemate	4.8	7.4	7.5	7.0	6.4
Ibuprofen	Racemate	~129	~131	~121	~117	~119
Dexibuprofen	*S*-enantiomer	0	0.05	0.5	0.5	0.4
Ketoprofen	Racemate	0.8	0.7	0.6	0.5	0.5
Dexketoprofen	*S*-enantiomer	0.03	0.02	0.02	0.01	0.01
Naproxen	*S*-enantiomer	26.2	29.2	29.3	32.0	43.5
Analgesics:						
Methadone	Racemate and *R*-enantiomer	1.0	1.2	1.4	1.6	1.8
Tramadol	2 isomers: *1R,2R*(+), *1S,2S*(-)	17.0	20.5	23.9	26.9	30.0
Codeine	(-)-enantiomer	25.2	24.3	30.2	35.2	37.9
Dihydrocodeine	(-)-enantiomer	10.9	11.4	11.3	11.2	11.0
Morphine	(-)-enantiomer	2.6	2.9	3.3	2.3	-

Table 7. Prescription of chiral drugs in England – selected chiral CNS drugs (approximate values)[19].

Chiral drug	Marketed as	Prescription [tonnes year^{-1}]				
		2004	2005	2006	2007	2008
Antidepressants:						
Fluoxetine	Racemate	3.6	3.6	3.8	4.1	4.2
Citalopram	Racemate	3.0	3.3	4.0	5.0	5.9
Escitalopram	*S*-enantiomer	0.4	0.5	0.6	0.5	0.5
Paroxetine	Single-enantiomer	1.9	1.6	1.5	1.4	1.3
Sertraline	*1S,4S*(+)-enantiomer	4.2	4.2	4.4	4.8	5.2
Trimipramine	Racemate	0.4	0.3	0.3	0.3	0.3
Venlafaxine	Racemate	10.3	9.0	7.7	7.7	8.2
Mirtazapine	Racemate	0.9	1.1	1.3	1.6	1.8
Bupropion	Racemate	1.1	1.1	1.0	1.0	0.5
Sedative/hypnotics:						
Zopiclone	Racemate	0.7	0.6	0.6	0.7	0.7
Diazepam	Racemate	0.7	0.7	0.7	0.7	0.7
Oxazepam	Racemate	0.1	0.1	0.1	0.1	0.1
Temazepam	Racemate	1.5	1.5	1.3	1.2	1.1
Antiepileptics:						
Ethosuximide	Racemic	0.7	0.7	0.6	0.6	0.6
Vigabatrin	Racemic	1.4	1.3	1.2	1.1	1.0
Levetiracetam	*S*-enantiomer	10.2	12.8	15.7	19.0	21.2
Entacapone	*E*-isomer	1.8	2.3	2.7	3.2	3.6

Table 8. Prescription of chiral drugs in England – selected chiral cardiovascular drugs (approximate values)[19].

Chiral drug	Marketed as	Prescription [tonnes year^{-1}]				
		2004	2005	2006	2007	2008
Beta-blockers:						
Atenolol	Racemate	42.2	41.6	37.7	32.3	30.1
Labetalol	Racemate	2.0	1.9	1.8	1.7	1.7
Metoprolol	Racemate	3.0	3.1	3.0	3.0	2.9
Propranolol	Racemate	8.0	7.9	7.7	7.6	7.7
Sotalol	Racemate	3.5	3.4	3.4	3.4	3.3
Anticoagulants:						
Warfarin	Racemate	0.7	0.8	0.8	0.9	0.9
Calcium channel blockers:						
Verapamil	Racemate	8.0	7.2	7.1	7.0	6.8
Diltiazem	*Cis* (+)-stereoisomer	24.1	24.3	24.3	24.4	24.4
Felodipine	Racemate	0.7	0.8	0.9	1.0	1.0
Amlodipine	Racemate	2.3	2.5	3.0	3.5	3.9
Anti-arrhythmic drugs:						
Disopyramide	Racemate	0.6	0.6	0.5	0.5	0.4
Flecainide	Racemate	1.5	1.5	1.6	1.7	1.7
Mexiletine	Racemate	0.1	0.1	0.1	0.1	0.09
Propafenone	Racemate	0.5	0.5	0.5	0.5	0.5
Angiotensin-Converting Enzyme Inhibitors:						
Ramipril	S-enantiomer	2.1	2.5	2.9	3.5	4.0
Enalapril	S-enantiomer	2.0	2.0	1.9	1.8	1.8
Lisinopril	S-enantiomer	3.4	3.6	3.9	4.1	4.6
Angiotensin-II Receptor Antagonists:						
Valsartan	S-enantiomer	5.7	6.8	7.7	8.2	8.3
Losartan	R-enantiomer	6.9	7.9	8.6	8.8	8.9
Lipid regulating drugs:						
Atorvastatin	Single-enantiomer	8.0	10.1	11.1	10.6	10.7
Simvastatin	Single-enantiomer	12.3	16.8	24.3	33.1	39.0
Pravastatin	Single-enantiomer	2.6	2.3	2.1	1.9	1.9

Table 9. Prescription of chiral drugs in England - respiratory and gastro-intestinal drugs (approximate values)[19].

Chiral drug	Marketed as	Prescription [tonnes year^{-1}]				
		2004	2005	2006	2007	2008
Bronchodilators (β2-agonists):						
Salbutamol	Racemate	0.3	0.3	0.2	0.1	0.2
Terbutaline	Racemate	0.03	0.03	0.02	0.01	0.008
Bambuterol	Racemate	0.006	0.005	0.005	0.004	0.003
Antihistamines:						
Cetirizine Levocetirizine	Racemate *R*(-)-enantiomer	0.9 0.07	0.8 0.08	0.9 0.08	1.0 0.06	1.2 0.05
Fexofenadine	Racemate	4.8	5.0	-	5.7	6.2
Proton pump inhibitors:						
Omeprazole Esomeprasole	Racemate *S*(-)-enantiomer	3.8 1.2	5.5 1.4	7.3 1.6	8.9 2.1	10.8 1.6
Pantoprazole	Racemate	1.0	1.1	1.1	0.9	0.7
Lansoprazole	Racemate	7.7	7.8	8.2	9.7	10.8